THE PHYSIOLOGY AND BIOCHEMISTRY OF THE *LAMINARIA PALLIDA/CARPOBLEPHARIS MINIMA* AND *ECKLONIA MAXIMA/SUHRIA VITTATA* ASSOCIATIONS FROM SOUTH-WESTERN CAPE WATERS, SOUTH AFRICA

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ABSTRACT

The two laminarian brown algae *Laminaria pallida* Grev. ex. J. Ag. and *Ecklonia maxima* (Osb.) Papenf. are important both economically and as major components of the South-western Cape waters, South Africa. Growing attached to these brown algae are several different species of red algae two of which, *Carpoblepharis minima* Bart. and *Suhria vittata* (L.) J. Ag., were chosen and the *L. pallida/C. minima* and *E. maxima/S. vittata* associations were studied using physiological and biochemical methods. *Carpoblepharis minima* has only been observed on *L. pallida*, whereas *S. vittata* has been found attached to various substrates as well as to *E. maxima*.

Prior to any investigation of the associations, certain physiological studies were undertaken on both brown and red algae. Translocation was found to occur in the brown algae at a velocity of 50-100mm h^{-1} in *L. pallida* and 240-300mm h^{-1} in *E. maxima* after incubation in seawater containing ^{14}C-sodium bicarbonate (1μCi $m\ell^{-1}$). Movement of ^{14}C-assimilates in *L. pallida* was slower than in *E. maxima* probably due to a difference in the medulla structure. Anatomical studies showed that *L. pallida* has trumpet shaped cells with single unconnected hyphae, whereas the hyphal threads of *E. maxima* were interconnected giving a more efficient translocation system. The only ^{14}C-labelled assimilates in the brown/red algal association were the acyclic polyol mannitol and certain amino acids. Mannitol plays a major role in the physiology of the Phaeophyceae and was the major translocant in *L. pallida* and *E. maxima*. Mannitol concentration varied seasonally in the frond of both brown algae; it, and the storage product laminaran, were at a maximum in winter and a minimum during summer, whereas the cell wall component alginic acid was at a higher concentration in summer than in winter. The ^{14}C-labelled amino acids may have been synthesised *in situ* from a ^{14}C-labelled precursor. Studies using ^{15}N-potassium nitrate showed that the major "free" amino

acids in *L. pallida* were alanine, glutamic acid and histidine, whereas in *E. maxima* they were alanine, glutamic acid and glutamine. The pathway of nitrogen incorporation was via the GS/GOGAT system.

The red algae contained mannitol and the major "free" amino acids were alanine, glutamine and histidine in *C. minima* and glutamine, glutamic acid and glycine in *S. vittata*. Both red algae were found to possess photosynthetic pigments, undergo ^{14}C-assimilation and to incorporate exogenously supplied mannitol. In both associations the major "free" amino acids were alanine, glutamic acid and glutamine. The major ^{14}C-labelled amino acid after photosynthesis was alanine.

There was transfer of the ^{14}C-labelled assimilates of photosynthesis from the brown to the red algal partner in the *L. pallida/C. minima* association, but this did not occur in the *E. maxima/S. vittata* relationship. *Carpoblepharis minima* was, therefore, considered to obtain nutrients from *L. pallida* but was also capable of producing its own products of photosynthesis, whereas *S. vittata* was found to be completely autotrophic and epiphytic upon *E. maxima*.

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CHAPTER 1

INTRODUCTION

The two brown algae, *Ecklonia maxima* (Osb) Papenf. (Phaeophyceae, Laminariales) and *Laminaria pallida* Grev. ex. J. Ag. (Phaeophyceae, Laminariales) dominate the kelp bed ecosystem of the South-western Cape waters, South Africa. They are also commercially important as a source of alginic acid, but little is known of either the physiology of these brown algae, or of the red algae which are associated with them. A critical appraisal by Evans *et al.* (1978) of the present knowledge of supposed red algal parasites, reveals that their taxonomy and taxonomic affinities with their host are not always fully understood. This is due, in part, to an over reliance on morphology and comparative structures and a lack of data derived from using modern physiological and biochemical techniques. In this investigation to be presented in Chapters 3, 4 and 5 two red algae *Carpoblepharis minima* Bart. (Rhodophyceae, Ceramiales) and *Suhria vittata* (L.)J. Ag. (Rhodophyceae, Nemalionales) were chosen due to the difference in their habitat. *Carpoblepharis minima* has only been observed on *L. pallida* whereas *S. vittata* has been seen attached to rocks and shellfish as well as on *E. maxima* (Simons, 1976). Fan and Papenfuss (1959) have reported *S. vittata* as a host plant with an attached red algal parasite. The red algae attached to the kelp have been termed "epiphytes" (Simons, 1976) although their true relationship with brown algae is unknown. Evans *et al.* (1978) suggested that the term "epiphyte" should be restricted to algae which are totally independent of the other partner for nutrients. Before an understanding of the algal associations could be made, investigations in determining translocation rates, carbohydrates and nitrogen physiology of *L. pallida* and *E. maxima* had to be undertaken.

1.1 Translocation of Assimilates

The translocation of assimilates in the Phaeophyceae is well documented (Bidwell *et al.*, 1972; Buggeln, 1977; Floch'h and

Penot, 1978; Hellebust and Haug, 1972; Lobban, 1978; Lüning *et al.*, 1972, 1973; Nicholson and Briggs, 1972; Parker, 1963. 1965, 1966; Parker and Huber, 1965; Sargent and Lantrip, 1952; Schmitz and Lobban, 1976; Schmitz *et al.*, 1972; Steinbiß and Schmitz, 1973). In previous studies the velocity of the translocate flow in kelp was determined by recording the furthest point of detectable radioactivity in the tissue divided by the duration of incubation in either ^{14}C-carbonate or bicarbonate (Floc'h and Penot, 1978; Hellebust and Haug, 1972; Nicholson and Briggs, 1972; Parker, 1965; Schmitz and Lobban, 1976; Schmitz and Srivastava, 1975; Schmitz *et al.*, 1972). Canny (1973) in a review on higher plants, has already suggested that the velocity derived in this manner was dependent on the initial dose of the isotope, i.e., the apparent velocity was greater at the higher levels of radioactivity. Buggeln (1976) claimed that a more important parameter of translocation than velocity was the rate of transport of organic matter; a method outlined by Parker (1971). This method involved measuring the dry weight increase in frond apices enclosed in black plastic bags. A general method for determining translocation velocity in members of the Laminariales with flat, thick blades, in this case *L. digitata* (Huds.) Lamour., has been devised by Buggeln (1981). Evidence of transport in *Macrocystis pyrifera* (L.) C. Ag. was shown by Parker (1965, 1966) and *Nereocystis luetkeana* (Mert.) Post. et Rupr. Schmitz and Lobban (1976) in a survey of thirteen species of Laminariales including *Laminaria setchellii* Silva; *M. pyrifera*, *M. integrifolia* Bory and *Alaria marginata* Post. et Rupr., have shown translocation velocities which ranged from 55 to 570 mm hr^{-1}. Lüning *et al.* (1973) found translocation occurred all year round in *L. saccharina* (L.) Lamour., but in *L. hyperborea* (Gunn.) Foslie translocation did not occur in October when no frond enlargement took place, or in January when the new frond was a tiny outgrowth. Floc'h and Penot (1972) have demonstrated the translocation of mineral elements in various members of the Laminariales and Fucales. The Laminariales are the largest and most structurally complex of the algae. In all portions of the thallus there are basically three types of tissue, a superficial meristoderm which is

both photosynthetic and meristematic, a parenchymatous cortex and a central medulla (Sideman and Scheirer , 1977). The innermost cells of the cortex give rise to two types of cells which contribute to the formation of the medulla (Schmitz and Srivastava, 1975). New sieve elements are continually produced and superimpose on one another to form "sieve tubes" running longitudinally in the thallus (Schmitz and Srivastava, 1975). Sieve elements are usually trumpet shaped due to differential tension (Sideman and Scheirer, 1977).

In the red alga *Delesseria sanguinea* (Huds.) Lamour., leucine-(U)-^{14}C was translocated at a maximum rate of 630 mm h^{-1} and the pathway is thought to be through conducting elements up to 540µm long which are interconnected by pit fields (Hartmann and Eschrich, 1969).

1.2 Carbohydrate Physiology

Parker (1965,1966) showed that ^{14}C-labelled assimilates in *Macrocystis* moved with velocities of 650 to 780 mm h^{-1} and the major ^{14}C-labelled products in the exudate were the acyclic polyol D-mannitol and some amino acids. A comprehensive review of polyols has been compiled by Lewis and Smith (1967a). Mannitol has been found to be universally present in the Phaeophyceae (Bidwell and Ghosh, 1963; Coassini Lokar and Baradel, 1967; Craigie, 1974; Mackie and Preston, 1974; Martinez Nadal *et al.*, 1963; Munda, 1967; Parker, 1966; Percival, 1968; Pompowski and Trokowicz, 1966; Rao, 1969; Schweiger, 1967; Stephen, 1980). Exogenously supplied mannitol was poorly utilized by *Fucus* L. (Bidwell and Ghosh, 1963) whereas endogenous mannitol was readily respired and interconvertible with laminaran in *Eisenia* Aresch. (Yamaguchi *et al.*, 1966). Drew (1969) found that in the metabolism of several ^{14}C-labelled sugars in eleven species of brown algae, no species would convert fructose or galactose to mannitol. The majority formed a glucan, presumably laminaran, from glucose and *Ascophyllum nodosum* (L.) Le Jolis and *Pelvetia canaliculata* (L.) Descaisne et Thuret changed both glucose and mannose to mannitol. The presence of certain enzymes suggested mannitol was formed

from mannitol-1-phosphate which may arise from the reduction of fructose-6-phosphate (Yamaguchi *et al.*, 1969).

Mannitol has a close relationship with two other important components of the kelp, the cell wall compound, alginic acid and the storage product, laminaran (Percival and McDowell, 1967). The generally accepted structure of alginic acid comprises a block co-polymer in which regions of 1,4-linked D-mannuronic acid, 1,4-linked L-guluronic acid and alternating D-mannuronic and L-guluronic acids occurred (Haug *et al.*, 1966, 1967a). The block content appeared to depend on the source; the chemical structure has been related to physical properties by Haug *et al.* (1967b). Laminaran is a term covering a range of β—1,3-linked glucans. Mannitol, laminaran and alginic acid showed a seasonal variation in the fronds of kelp, when mannitol and laminaran content were high alginic acid was low and vice versa (reviewed by Percival and McDowell, 1967). In studies on *L. pallida* and *E. maxima* from South-western Cape waters alginic acid content rose from a minimum in winter to a maximum in summer, whereas the converse was true of mannitol and laminaran concentration (von Holdt *et al.*, 1955). Only the frond of *E. maxima* contained laminaran in any quantity (von Holdt *et al.*, 1955). Hay *et al.*, (1983) demonstrated a seasonal variation in algin content in the blade of *E. maxima* but no variation in the stipe of *E. maxima* nor in the blade or stipe of *L. pallida* or *Macrocystis angustifolia* Bory. Kremer (1978a) found that the main accumulated products of photosynthetic CO_2 fixation vary in the red algae. *Porphyra umbilicalis* (L.) J. Ag. and *Chondrus crispus* Stackh., showed an almost exclusive carbon assimilation by photosynthesis via ribulose-1,5-diphosphate carboxylase. *Corallina officinalis* L., *Palmaria palmata* (L.) Stackh. and *Gigartina stellata* (Stackh.) Batt., accumulated ^{14}C in the neutral compound floridoside (=2-O-glycerol-α-D-galactopyranose). *Delesseria sanguinea*, *Ceramium rubrum* (Huds.) C. Ag., and *Rhodomela confervoides* (Huds.) Silva, representing members of the Delesseriaceae, Ceramiaceae and Rhodomelaceae, respectively, do not photosynthesize floridoside but show intense ^{14}C-labelling in an acidic constituent, mannosidoglycerate (=digeneaside) reported for the first time as a

rapidly ^{14}C-labelled and accumulated photosynthate in a variety of red algal species in the Ceramiales. The marine Rhodophyceae in general differ from other autotrophic plants in lacking free mono or oligosaccharides and instead contain the low molecular weight compound floridoside (Kremer, 1978a). Kremer (1978b) has found the heteroside, floridoside, and the dissacharide, trehalose, to be the most abundant end products of photosynthetic carbon reduction in three species of freshwater Rhodophyceae.

Craigie (1974) reported that in numerous red algae mannitol has been identified but it rarely accumulates except in the Ceramiales where floridoside is a minor reserve product. Two other polyols, dulcitol and sorbitol, have been identified in a marine red alga *Bostrychia scorpioides* (Huds.) (Craigie, 1974). Kremer (1976a) in a reappraisal of mannitol in the Rhodophyceae, found an absence of mannitol and any other hexitols as a ^{14}C-assimilate and concluded that it may have been incorrectly identified. Mannitol was found in the thallus of red algae in some forms of epiphytic relationship where severe alterations in the normal assimilation patterns may have occurred (Kremer, 1976a). Stephen (1980) in a review article on plant carbohydrates, states that the distinctive groups of polysaccharides found widespread among Rhodophyceae consist of modified, sulphated D-galactans with α 1→3 and β 1→4 linkages. Representative species within a family are found to contain one or other of two groups, D- and DL-galactans termed carrageenans and agars respectively (Stephen, 1980). Some members of the Rhodophyceae contain neutral 1→4 linked β-D-mannans and β-D-xylans which are skeletal in function (Stephen, 1980).

1.3 Nitrogen Metabolism

The majority of investigations into nitrogen metabolism have been on bacteria, higher plants and, to a lesser extent, on unicellular algae with little on macroalgae. Haxen and Lewis (1982) have studied nitrate assimilation in *Macrocystis angustifolia*. The two most common sources of nitrogen used for the growth of unicellular algal culture have been nitrate and ammonium ions. If both were supplied to algae the nitrate

was not utilized until all the ammonium ions had been taken up (Syrett, 1962). It has been generally accepted that nitrate-N is reduced to the ammonium level before being incorporated into organic compounds (Morris, 1974; Syrett, 1962). Recent work with higher plants (Beevers and Hageman, 1969; Hewitt, 1970) and algae (Aparichio *et al.*, 1971; Hattori and Meyers, 1966, 1967; Zumft *et al.*, 1969) demonstrated that two enzymes catalysed the reduction of nitrate to ammonium-N. The first, nitrate reductase (E.C.1.6.6.1.) catalysed the reduction of nitrate to nitrite while the second enzyme, nitrite reductase (E.C.1.6.6.4.) catalyses the reduction of nitrite to ammonia. Nitrate reductase was first reported in algae in extracts of *Chlorella* Beijerinck (Schafer *et al.*, 1961) and various workers have analysed the activity of this algal enzyme (Aparichio *et al.*, 1971; Cárdenas *et al.*, 1971; Losada *et al.*, 1965, 1970; Osajima and Yamafuji, 1964; Vega *et al.*, 1971). Although nitrate reductase has been measured in other algae, including natural phytoplankton populations (Eppley *et al.*, 1969, 1970) the work done so far has yielded little information on the precise nature of the enzyme and the mechanism of its action in algae (Morris, 1974). Czygan (1963) first measured the activity of nitrite reductase in algae in extracts of *Ankistrodesmus braunii* (Naeg.) Brunn. and suggested the presence of two nitrite reductases. One of these, found on large particles, was dissimilatory, and the other, which required high energy phosphate was localised on smaller particles, was assimilatory. These results, however, have not been substantiated (Morris, 1974). The major pathways of reduced nitrogen assimilation in higher plants were demonstrated; a glutamate dehydrogenase (GDH, E.C.1.4.1.3.) (Sims and Folkes, 1964) and a glutamine synthetase/L glutamate:$NADP^+$ oxidoreductase (transmitting) pathway (GS, E.C.6.3.1.2./GOGAT, E.C.1.4.7.1.) (Lea and Miflin, 1974). The net result of the two pathways was the same. Recent evidence has supported the hypothesis that the principal pathway is via the GS/GOGAT system and this has been summarised in reviews by Lea and Miflin (1979), Lea and Norris (1976) and Miflin and Lea (1976, 1977). Glutamate dehydrogenase was shown to be important in fungi (Sims and Folkes, 1964)

and in the green, marine alga *Caulerpa* Lamour and the presence of GDH (with a low Km) which could provide a direct route to glutamate was demonstrated (Gayler and Morgan, 1976). It appears, however, to play no role in the nitrate assimilation of higher plants (Kaiser, 1978; Kaiser and Lewis, 1980; Lewis and Probyn, 1978; Probyn, 1978; Probyn and Lewis, 1979). The pathway of ammonium ion assimilation via GS/GOGAT activity in chloroplasts is shown by the following equations:

$$\text{Glutamate} + NH_4 + ATP \rightarrow \text{Glutamine} + ADP + Pi + H_2O$$

$$\text{Glutamine} + \text{2-oxoglutarate} + FdH \rightarrow 2 \text{ glutamate} + Fd$$

1.4 Algal Associations

A complete gradient of forms exists in the red algae in which the relationship ranges from epiphytism to one of a more parasitic nature. Adelphoparasites are those red algal parasites which are closely related to their host and those which are unrelated are termed alloparasites (Feldman and Feldman, 1958). About 90% of the adelphoparasites belong to the same family or order as their hosts (Dawson, 1966). Setchell (1918) suggested that the similarity could be accounted for if the parasites had mutated from host progeny. Fan (1961) claimed that Setchell's (1918) example of *Agardhiella tenera* J. Ag. was unique, but Andrews (1976) considered there were too many genera of adelphoparasites for mutation to account for all of them. Certain red algal alloparasites may have originated from epiphytes which gradually penetrated the host tissue establishing metabolic dependence (Sturch, 1926). One piece of evidence supporting Sturch's (1926) hypothesis is the formation of secondary pit connections between the host and the red algal parasite and their presumed function in the transfer of nutrients. Fan (1961) amplified Sturch's hypothesis to include adelphoparasitic origin and Andrews (1976) accepted that this was generally consistent with available evidence. Depending on their stage of epiphytic origin, red algal parasites may display a different vegetative area and nutritional dependence on their host (Andrews, 1976). Many red algal epiphytes are superficially attached to their host (Dixon, 1973; Fritsch, 1945) and it has been possible to grow

a red algal epiphyte upon an artificial substrate (Harlin, 1973a). Despite this superficial attachment, ^{32}P has been demonstrated to move from host to epiphyte and, to a lesser extent, from epiphyte to host (Harlin, 1973b; Linskens, 1963). Harlin (1973b) has demonstrated the transfer of ^{32}P and ^{14}C from two species of sea grass, *Phyllospadix scouleri* Hook. and *Zostera marina* L. and the red alga *Smithora naiadum* (Anderson) Hollenb. attached to the sea grasses. Both ^{32}P and the products of ^{14}C-assimilation were found to be translocated from host to epiphyte and epiphyte to host. Part of the transfer occurred through the host and part by leakage from the host into the medium and subsequent uptake by the algae. Although blades of *S. naiadum* would appear to be obligate epiphytes their growth on artificial substrate under field conditions has been reported (Harlin, 1973a). Two examples of host/epiphyte relationships which show that morphology gives little indication of the nutritional dependence of the epiphyte on the host are *Smithora naiadum*, attached to sea grasses (Harlin, 1973b) and *Polysiphonia lanosa* (L.) Tandy growing on *Ascophyllum nodosum* (Evans *et al.*, 1978). The presence or absence of rhizoids does not indicate the nutritional status of the epiphyte. *Smithora naiadum* has no rhizoids penetrating the hosts, *Z. marina* and *P. scouleri*, but produces a flat, basal cushion offering a large adhering surface; movement of ^{32}P and ^{14}C from host to epiphyte and from epiphyte to host was demonstrated (Harlin, 1973b). In another example, the rhizoids of *P. lanosa* digested their way into the host *A. nodosum* and host wall material was incorporated into the outer wall of the rhizoid (Rawlence, 1972). Studies have failed to demonstrate large scale directional translocation of photoassimilates within *A. nodosum* or between this alga and the epiphyte *P. lanosa* (Harlin and Craigie, 1975; Turner and Evans, 1977). Turner and Evans (1978) have demonstrated that translocation can occur within *P. lanosa* and there may be an exchange of nutrients on a limited scale at the interface between *P. lanosa* and *A. nodosum*.

In some morphological studies a clear differentiation between the host and epiphyte tissue could not be established due

to displacement of host cells. This was found in *Choreocolax polysiphoniae* Reinsch growing on *P. lanosa* where transfer of sodium mannoglutarate from host to epiphyte was thought to have occurred (Callow *et al.*, 1979). A limited number of red algae exhibit a more intimate relationship either by a superficial contact or with an endophytic attaching system penetrating the host to various depths thereby causing cell destruction. In New Zealand, *Herpodiscus* Lindauer, a brown algal parasite, caused complete destruction of the host tissue *Durvillaea antartica* (Chamisso) Hariot and the entire lamina of the host was lost in some cases (South, 1974). Members of the Florideophycidae provide the best examples of parasites which both penetrate the host and exhibit vegetative reduction. Good examples are to be found in the genera, *Ceratocolax* Rosenv., *Ricardia* Derb. et Solier and *Janczewskia* Solms-Laubach. *Ceratocolax* may either invade the medulla and kill cells, or penetrate to just below the surface sending haustoria into the walls of the underlying cells. Pigmentation in these genera varies from being totally absent to being abundant, but both pigmented and non-pigmented species have been recorded within the same genus, e.g., *Janczewskia*. Martin and Pocock (1953) suggested that, in some cases, pigmentation of otherwise colourless parasites might result from the leaching of host pigments into the parasite when removed from the natural marine environment. Obviously, if no pigments are present there must be a substantial dependence by the organism on the host, but where pigments are present the degree of dependence on the host is not clear. Evans *et al.* (1978) in a review, claimed an association could still be parasitic if there was a dependence requiring minor metabolites, growth factors or vitamins. The degree of parasitism may vary with the stage of development; for example, the young stages of *Janczewskia morimotoi* Tokida growing on *Laurentia nipponica* Yamada are non-pigmented and contain proplastids. After three weeks of growth pigment develops and chloroplasts are present, which strongly suggest it is parasitic at an early stage of its development (Nonomura, 1979). The red alga, *Polysiphonia lanosa* possesses a very complex system of rhizoids penetrating

deeply into the host. *Ascophyllum nodosum* (Rawlence and Taylor, 1970). The studies of Harlin and Craigie (1975) and Turner and Evans (1978) have failed to demonstrate active intercellular transport of photoassimilated products within *A. nodosum* or between this plant and the epiphyte of *P. lanosa*. The evidence for the lack of translocation in *A. nodosum* tissue suggests that *P. lanosa* would only be able to obtain photoassimilatory products from cells around the rhizoids (Turner and Evans, 1977). Translocation has been shown to occur in *P. lanosa* so that an effective distribution of any metabolites derived from *A. nodosum* could occur within the epiphyte (Turner and Evans, 1978). *P. lanosa* contains several water soluble sugars but only sodium mannoglycerate showed the presence of ^{14}C-label after a one hour pulse in ^{14}C-bicarbonate (Turner and Evans, 1977). In the case of *Holmsella* Sturch growing on the red alga *Gracilaria verrucosa* (Huds.) Papenf. the alcohol glycoside, floridoside, was shown to be the major photosynthate as ^{14}C-assimilates were being transferred from *Gracilaria* to *Holmsella* and accumulated in the recipient as floridoside, mannitol and starch (Turner and Evans, 1977). Goff (1976) has shown that ^{14}C-assimilated by the host plant *Odonthalia floccosa* (Esper.) Falkenberg, was transferred primarily from medullary cells to the adjacent rhizoids of the alloparasite *Harveyella mirabilis* (Reinsch) Schmitz et Reinke. Goff and Cole (1976) have shown the initial germination of *Harveyella* to occur in host wounds caused by grazing isopods and amphipods. Rhizoids penetrate between the cells and establish secondary pit connections (Goff, 1976) with the host cell. The rhizoidal cells of *Harveyella* grow rapidly within the host cells causing rupturing of the host's outer wall and development of the emergent colourless parasite.

1.5 Studies in South-western Cape Waters

Several investigations have recently been undertaken, and are continuing, to obtain a complete picture of the ecosystem of the South-western Cape waters. Some of the studies have already been published, viz., the phytoplankton (Barlow, 1980, 1981; Brown, 1980) the growth and primary productivity

of the kelp (Dieckmann, 1978, 1980; Mann *et al.*, 1979) the biomass and structure of the kelp communities (Field *et al.*, 1977, 1980a; Velimirov *et al.*, 1977) upwelling and its biological implications (Field *et al.*, 1980b) and fragmentation, release of nutrients from the kelps and associated microbial communities (Linley *et al.*, 1981; Lucas *et al.*, 1981; Newell *et al.*, 1980; Stuart *et al.*, 1981). An outline of the complete energy balance in the kelp community has been produced by Newell *et al.* (1982).

The data presented in this thesis concern the physiology and biochemistry of *Laminaria pallida* and *Ecklonia maxima*. This includes determining translocation rates, identifying the products of translocation, analysing the seasonal variations in mannitol, laminaran and alginic acid and elucidating the pathway of nitrogen assimilation. Only the studies of von Holdt *et al.* (1955) and Hay *et al.* (1983) exist on *L. pallida* and *E. maxima*. Growing in association with these kelp are various red algae and the relationship between *L. pallida/Carpoblepharis minima* and *E. maxima/Suhria vittata* was investigated to establish whether the red alga receives any metabolites from its brown algal partner.

CHAPTER 2

MATERIALS AND METHODS

2.1 Collecting Site and Plant Material

Ecklonia maxima and *Laminaria pallida*, both with and without attached red algae, were collected at Oudekraal (33°59'S, 18°21'E) on the Atlantic seaboard of the Cape Peninsula (Fig. 1). This site was initially chosen by an interdisciplinary group of scientists for a kelp bed ecosystems project (Field *et al.*, 1977) and has a temperate climate with strong south-easterly winds especially during spring and early summer. During winter it is pounded by heavy swells caused by northerly winds.

Ecklonia maxima and *L. pallida* are classified in the Laminariales, a group containing some of the structurally most complex algae, and individually members are commonly termed kelp plants. *Laminaria pallida* and *E. maxima* have cylindrical, smooth stipes which terminate in a flat lamina. The meristematic region is situated in the transition zone between stipe and lamina. The stipe of *E. maxima* is hollow and enlarged to form an air bladder at the top, unlike the solid stipe with no air bladder of *L. pallida*. Dieckmann (1978) related the length of the stipe of *L. pallida* to the age of the sporophytes. One metre plants would be approximately one year old when collected at a depth of eight metres and two and a half years old from a depth of fourteen metres, the plants in deeper water being slower growing. In order to reduce errors due to changes in metabolism with age, all plants selected for this study were about one metre sporophytes collected at a depth of four to eight metres under a canopy of mature kelp. This was the maximum size that could be accommodated in the perspex chambers used in the ^{14}C-assimilation experiments (see Fig. 8). Entire plants were transported to the laboratory in buckets of seawater immediately after collection. Some plants, which secreted copious quantities of mucilage, were discarded.

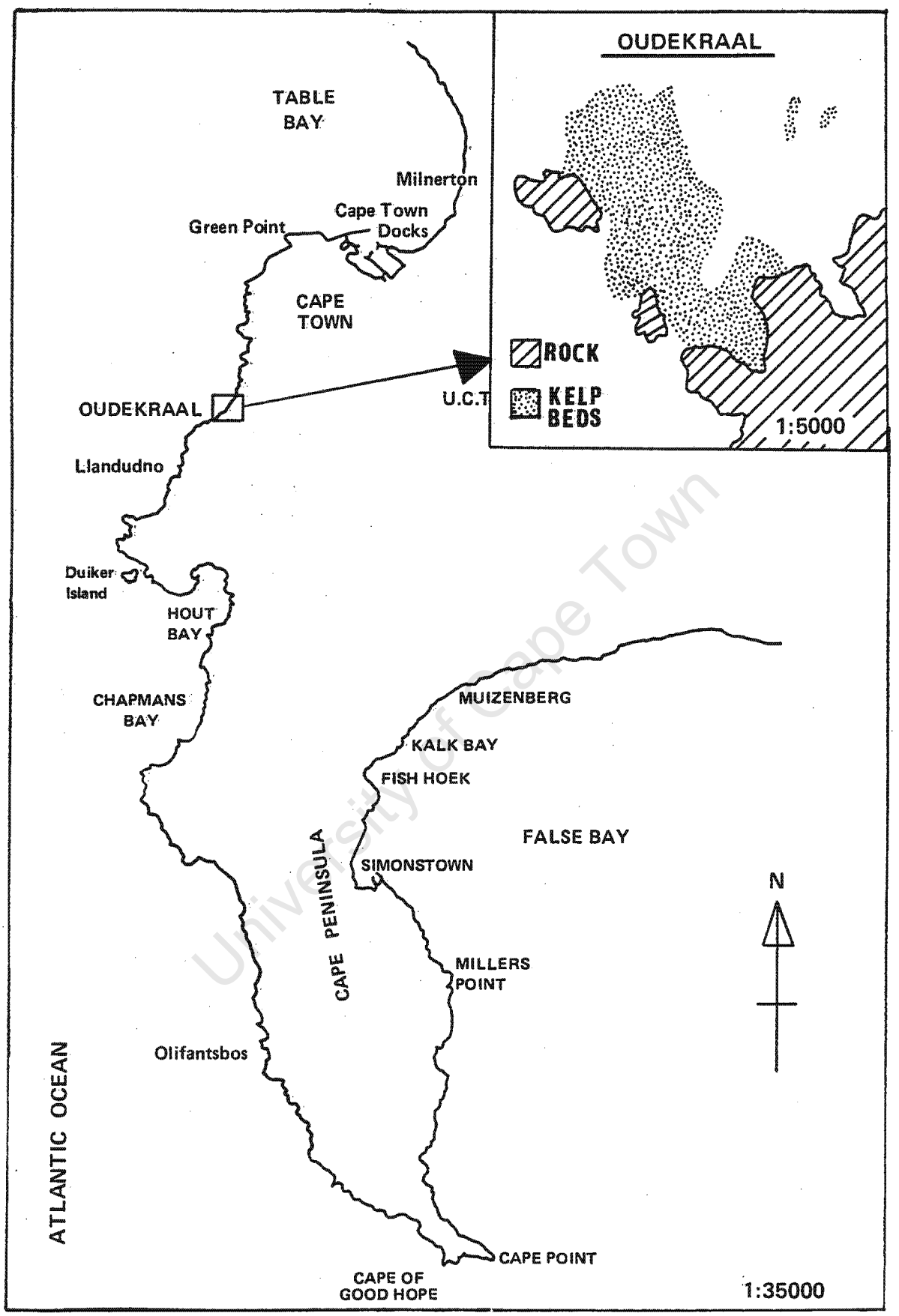


FIG.1

The Cape Peninsula showing the Oudekraal collecting site and kelp bed distribution at Oudekraal (after Dieckmann, 1978)

Attached to the brown algae are various species of red algae. The two species chosen for this investigation were *Carpoblepharis minima* attached to *L. pallida* (Pl.1A) and *Suhria vittata* (Pl.1B) attached to *E. maxima*.

2.2 ^{14}C-Incorporation in *Laminaria pallida* and *Ecklonia maxima*

Sporophytes of *L. pallida* and *E. maxima* were exposed to ^{14}C in different types of experiments but in all cases the isotope used was sodium ^{14}C-bicarbonate ($NaH^{14}CO_3$) obtained as a sterile, aqueous solution from the Radiochemical Centre, Amersham, Bucks., England. The seawater was millipore filtered (0,45 µm) and all experimental plants were placed in buckets of filtered seawater in a Conviron Model E15 Growth Chamber for a minimum of two hours to equilibrate prior to any experiments. All sporophytes were subjected to 93 µE $m^{-2}s^{-1}$ at the plant surface emitted by Sylvania (Canada) cool white, high intensity, fluorescent tubes supplemented with 60W incandescent lamps at 10°C and 80% humidity for six hours. During the preliminary studies to determine translocation rates in the kelp, a round perspex ampoule (50mm external and 37mm internal diameter) was attached to the fronds of six different plants of both species and the apparatus assembled as illustrated in Schmitz *et al.* (1972). The ampoule was attached using Sico-met 50 glue (Sichel-Werke Gmbh. Hanover) and 1 µCi ml^{-1} $NaH^{14}CO_3$, made up in filtered seawater, was introduced into it. Plants were incubated for two hours with the radioactive label, during which time they lay in large plastic trays completely submerged in filtered seawater. After two hours the ampoule was drained and removed, the plants washed in filtered seawater and left for a further hour in filtered seawater. Six plants of *L. pallida* and *E. maxima* were treated in this manner and Figs. 2-4 and 5-7 respectively show the point of attachment of the perspex ampoule and the sites of sampling. The lamina was punched with a 16mm diameter cork borer, the first sampling point being in the centre of the area covered by the ampoule and subsequent samples taken at fixed distances from this site.

The first two sampling sites in *L. pallida* were 10mm apart

PLATE 1

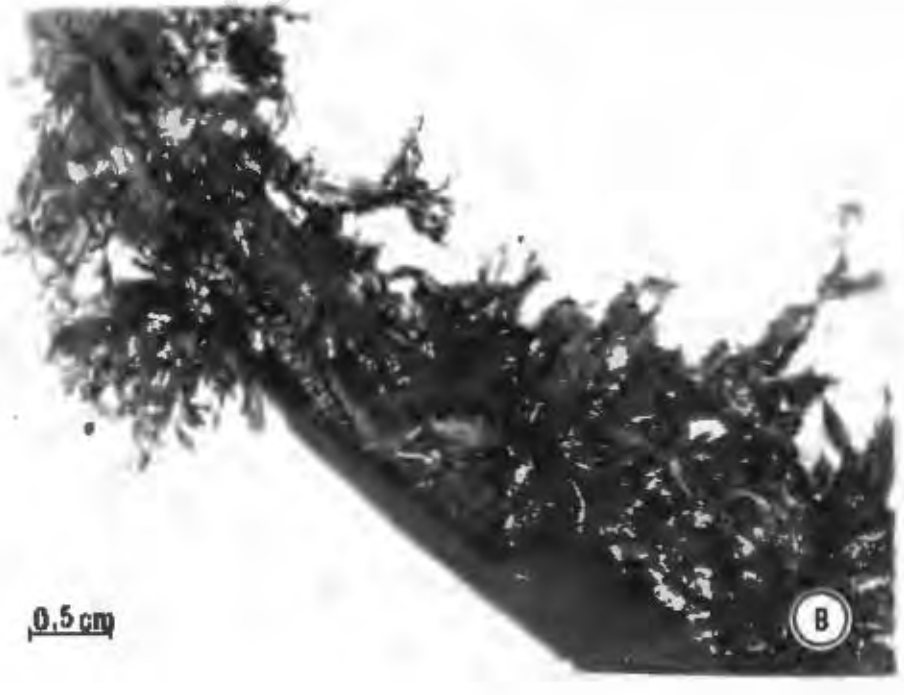
A

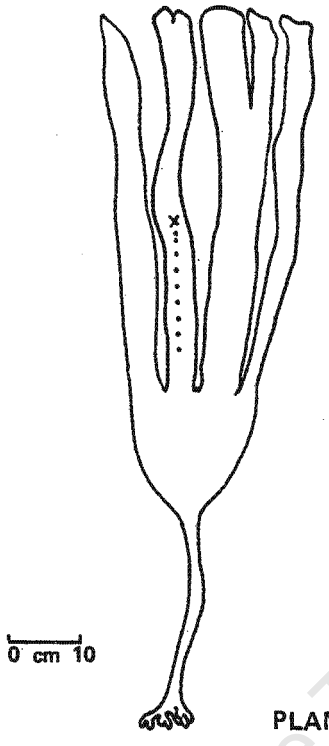
Carpoblepharis minima attached to the stipe of *Laminaria pallida*

B

Suhria vittata attached to the stipe of *Ecklonia maxima*

PLATE 1





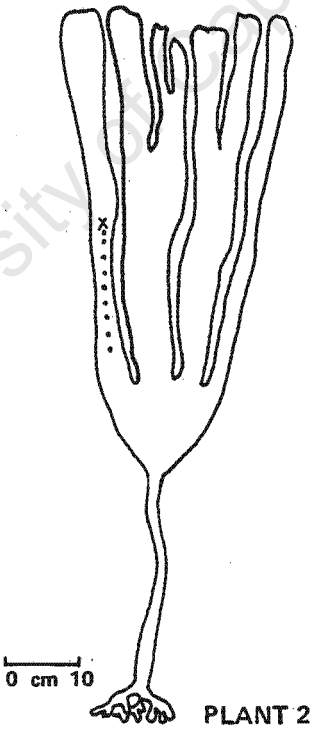
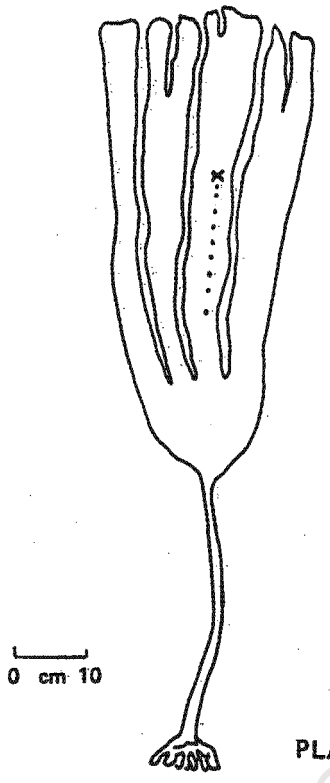


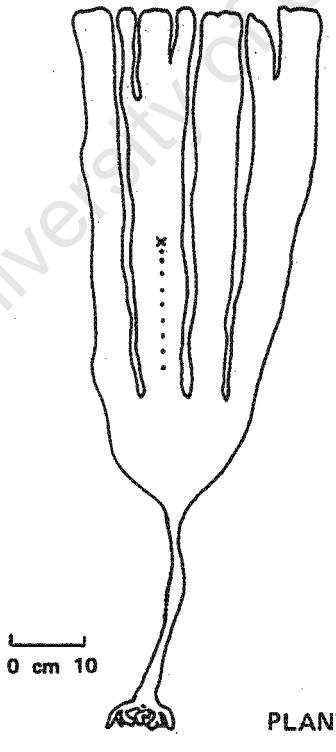
FIG.2 *L. pallida:* plants used in the translocation study

(x) denotes the site of attachment of the perspex ampoule

(•) the site of sampling



PLANT 3

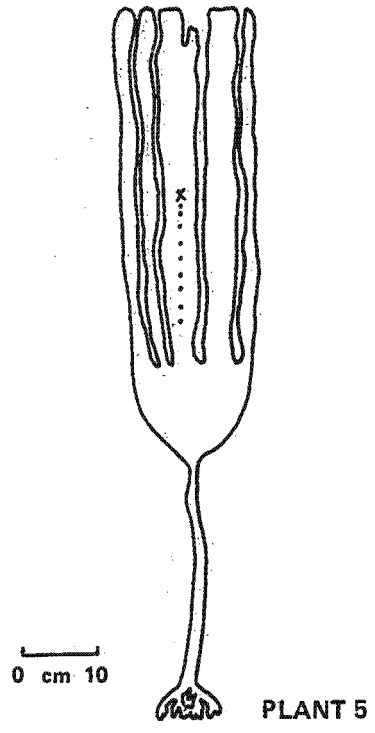


PLANT 4

FIG.3 *L. pallida:* plants used in the translocation study

(x) denotes the site of attachment of the perspex ampoule

(•) the site of sampling



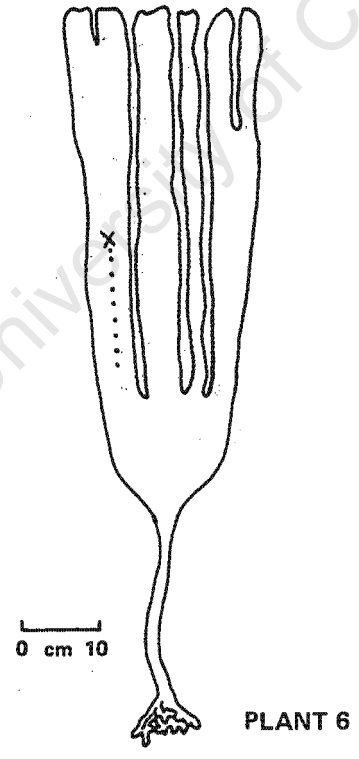
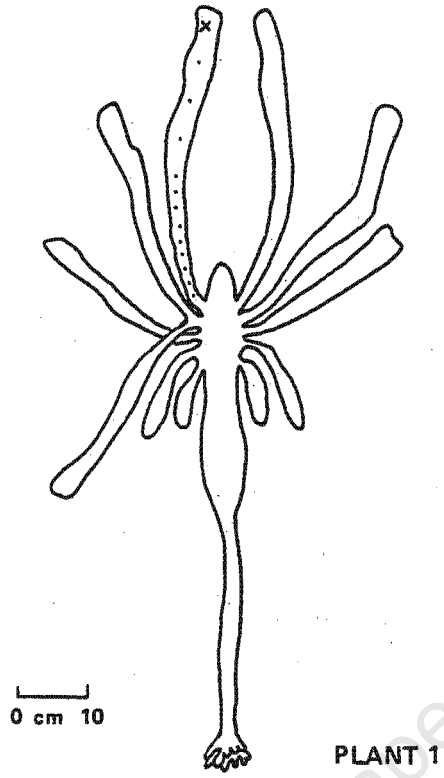


FIG.4 *L. pallida:* plants used in the translocation study
(x) denotes the site of attachment of the perspex ampoule
(•) the site sampling



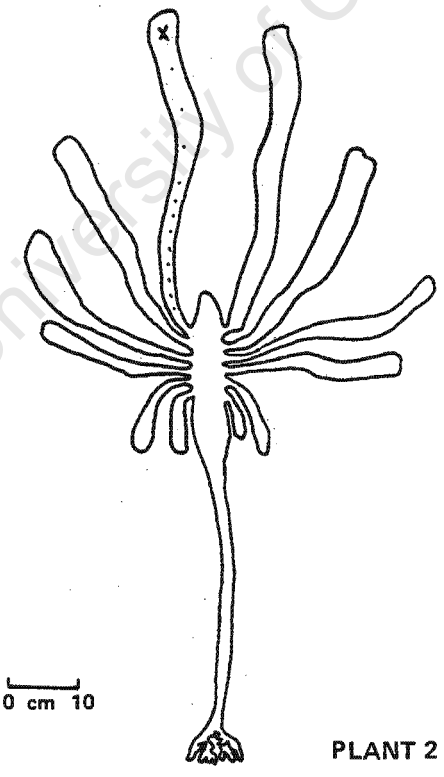
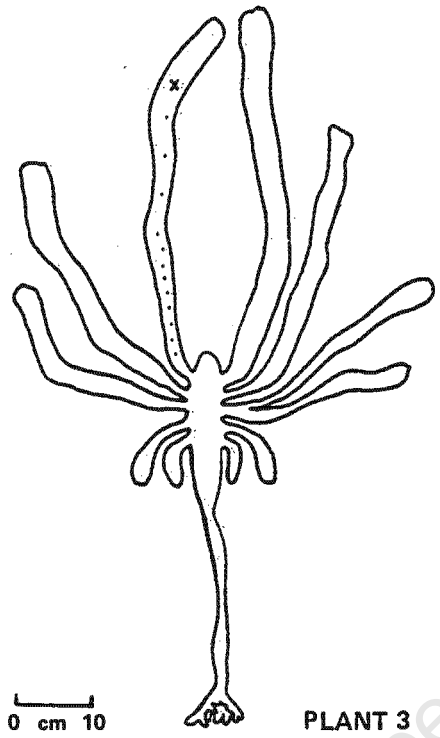


FIG.5 *E. maxima:* plants used in the translocation study

(x) deontes the site of attachment of the perspex ampoule

(•) the site of sampling



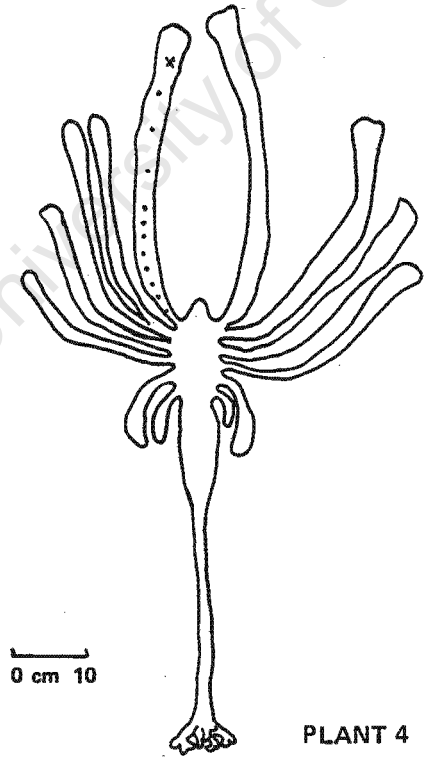
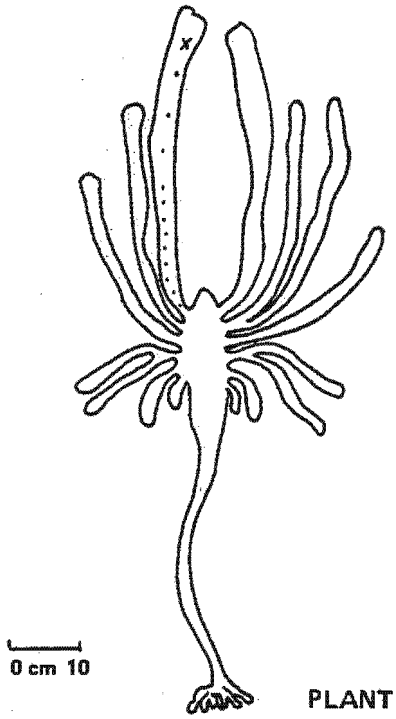


FIG.6 *E. maxima:* plants used in the translocation study

(x) denotes the site of attachment of the perspex ampoule

(•) the site of sampling



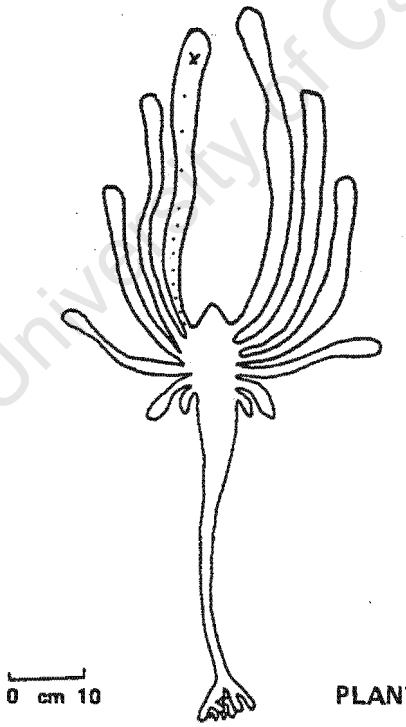


FIG. 7 *E. maxima:* plants used in the translocation study

(x) denotes the site of attachment of the perspex ampoule

(•) the site of sampling

and subsequent sites were at 20mm intervals. Samples were taken every 50mm for the first 200mm, then every 20mm for subsequent samples in the case of *E. maxima*.

In the majority of ^{14}C experiments it was necessary to expose only the frond to ^{14}C-label in isolation from the stipe so that the stipe and any attached red algae could subsequently be analysed for the presence of ^{14}C-assimilates, thus demonstrating translocation and transfer from the frond. In these experiments plants about one metre in length were placed in a specially designed perspex chamber (Fig. 8). The top and bottom halves of the chamber were separated by means of a neoprene rubber seal and the holdfast of the plant could be pushed through a small hole in the neoprene which ensured a water tight seal around the stipe. As a further precaution, Sico-met 50 glue was placed in the region between the stipe and the neoprene rubber. When preparing the chamber, neoprene rubber was cut to fit a specific plant and was fitted at the junction of the stipe and the frond. Figure 8 shows that when the top and bottom parts of the chamber were attached they formed a cylinder. The stipe was placed in the bottom half of the chamber which was completely filled with filtered, non-radioactive seawater. The neoprene rubber formed a seal across the top of the bottom chamber. The top half of the chamber was slipped over the lamina of the kelp and bolted to the bottom half with butterfly nuts. The neoprene rubber was smeared with silicon grease to ensure a water tight seal. The top half of the chamber was filled with seawater by opening both taps (see Fig. 8), attaching a rubber tube to the bottom tap and siphoning in seawater containing the ^{14}C-bicarbonate label (1 $\mu Ci\ m\ell^{-1}$). The taps were closed and the whole apparatus placed in the growth chamber lying on its side to provide even illumination of the plant by the overhead lighting and the seawater agitated by mechanical stirrers. After six hours the plants were removed, the frond and stipe separated and washed in several changes of fresh seawater to remove excess isotope. Twelve plants of *L. pallida* and *E. maxima* were treated in the above manner, with six samples (each 1,0g fresh mass) taken from each region (frond, stipe, holdfast and red algae).

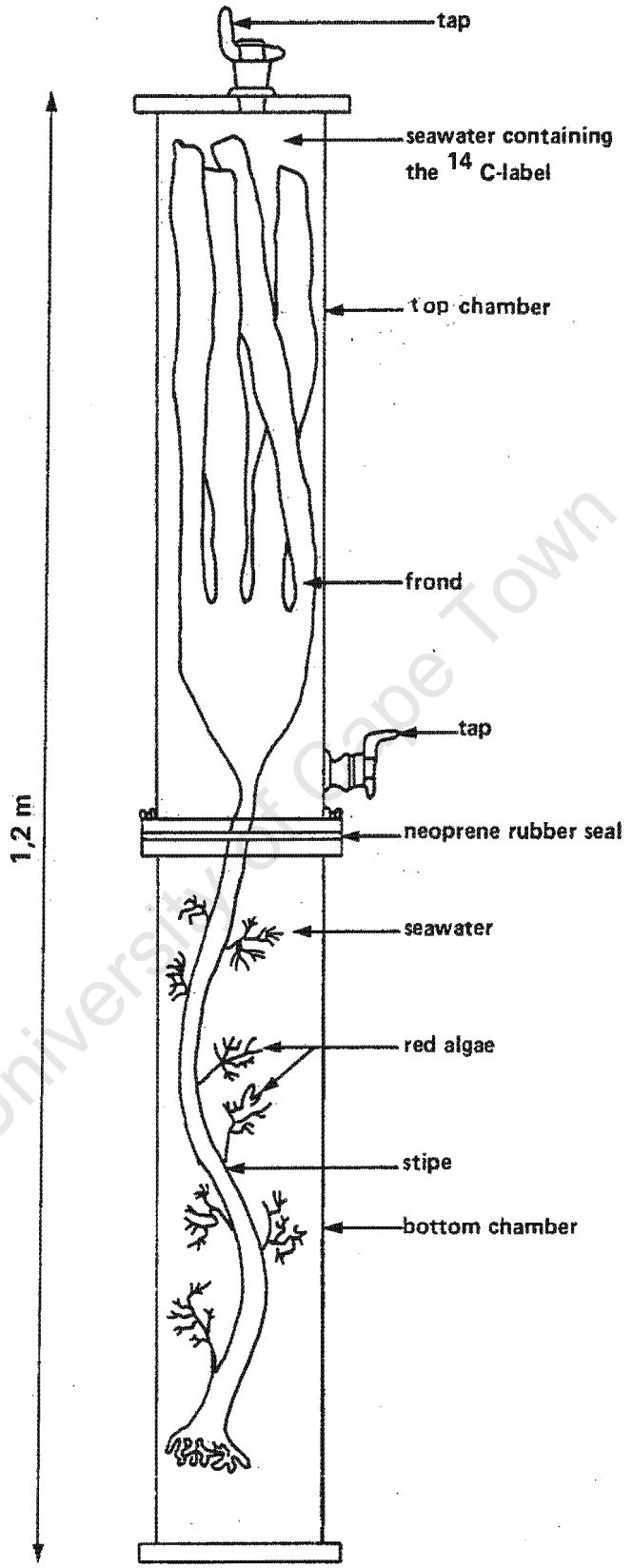


FIG.8 A diagrammatic sketch of the perspex chamber which enabled the frond to be separated from the stipe by means of a water tight neoprene rubber seal.

All samples were oven-dried at 65°C overnight.

The total tissue fraction was obtained by solubilizing cores of tissue using the method of Lobban (1974). The ethanol-soluble fraction was extracted by boiling under reflux for thirty minutes in three changes of 80% ethanol and the washings bulked. The laminaran fraction was extracted from coarsely milled samples (4mm mesh diameter) by the method of Black *et al.* (1951) which extracts 90% of the laminaran although it is contaminated by fucoidan. Alginic acid was isolated by the method of Mateus *et al.* (1977) and involved successive precipitations from different solvents to remove impurities. The residue fraction was that portion remaining after removal of the ethanol-soluble, laminaran and alginic acid fractions, and this was solubilized using the method of Lobban (1974). All fractions were evaporated to dryness and 10mℓ of Dimilume-30 (Packard Instrument Corporation Inc., 2200 Warrenville Road, Downers Grove, Illinois 60515) was added. Dimilume-30 cocktail has been specifically formulated to eliminate chemiluminescence caused by the presence of either alkaline material or peroxides in the samples. Radioactivity was monitored on a Beckman LS liquid scintillation spectrophotometer for twenty minutes. All results were converted to disintegrations per minute (DPM) after correcting for quenching using the external standard ratio and ^{14}C-toluene as a standard. These results in DPM g^{-1} dry mass from the ^{14}C-isotope experiments using the perspex chambers (Fig. 8) could be expressed in several ways. The major objective was, however, to demonstrate the transport of carbon containing compounds from the frond to other regions of the plant and then finally transfer to the red algae. It was decided to express results relative to the total radioactivity exported from the fronds after exposure to ^{14}C-bicarbonate. The percent ^{14}C-label translocated or exported was calculated using the following formula:

$$\text{Percent } ^{14}\text{C-label translocated} = \frac{^{14}\text{C -label in stipe + holdfast (+ red algae)}}{\text{Total } ^{14}\text{C-label recovered in whole plant}} \times 100$$

The percent per region (frond, stipe, holdfast and red algae) was calculated by the following formula:

$$\text{Percent } {}^{14}\text{C-label in a region} = \frac{\text{total } {}^{14}\text{C-label in a region}}{\text{total } {}^{14}\text{C-label recovered in the whole plant}} \times 100$$

2.3 Physiological Studies on *L. pallida* and *E. maxima*

Plants were subdivided into three regions (frond, stipe and meristematic region) in the seasonal studies and in the other experiments samples were taken from the frond, stipe and attached red algae. In all cases the plant was cut into small pieces, plunged into 80% ethanol and homogenised in an Ultra-Turrax type TP 18/10 homogeniser for three minutes. After being refluxed in 80% ethanol for ninety minutes (three changes of ethanol were made at thirty minute intervals) the ethanol extracts were bulked, dried and made up to a known volume in distilled water. The samples were further purified by partial deproteinization (Somogyi, 1945) and deionized by the addition of activated resins, Amberlite IR 120 (H) and IR (OH). The ethanol-soluble fraction was analysed for total sugars using the method of Dubois *et al.* (1956). The absorbance of the orange/yellow colour was measured on a Pye-Unicam SP 1800 ultra-violet spectrophotometer at 490nm against a water blank. The concentration of sugars was determined from a calibration curve for glucose (Fig. 9). Mannitol concentration was determined in the ethanol-soluble fraction by the periodate oxidation method (Lewis and Harley, 1965; Lewis and Smith, 1967b). Cameron *et al.* (1948) reported that glucose and other sugars were not readily oxidised by periodate under acidic conditions, whereas oxidation of mannitol was almost complete after one minute. Exactly one minute after the addition of 1mℓ M-acetate buffer (pH 4,5) and 1mℓ sodium metaperiodate (750mg ℓ^{-1}) the absorbance was read at 260nm on a Pye-Unicam SP 1800 ultra violet spectrophotometer. It was found that the absorbance, when compared with a water blank, was linear in the range 10-100 g $m\ell^{-1}$ mannitol (Fig. 10). In the seasonal variation studies mannitol concentration was obtained by the periodate oxidation method, laminaran by the method of Black *et al.* (1951) and alginic acid by the method of Mateus *et al.* (1977). The freeze dried alginic acid was weighed. A comprehensive survey of other methods of determining alginic acid is given

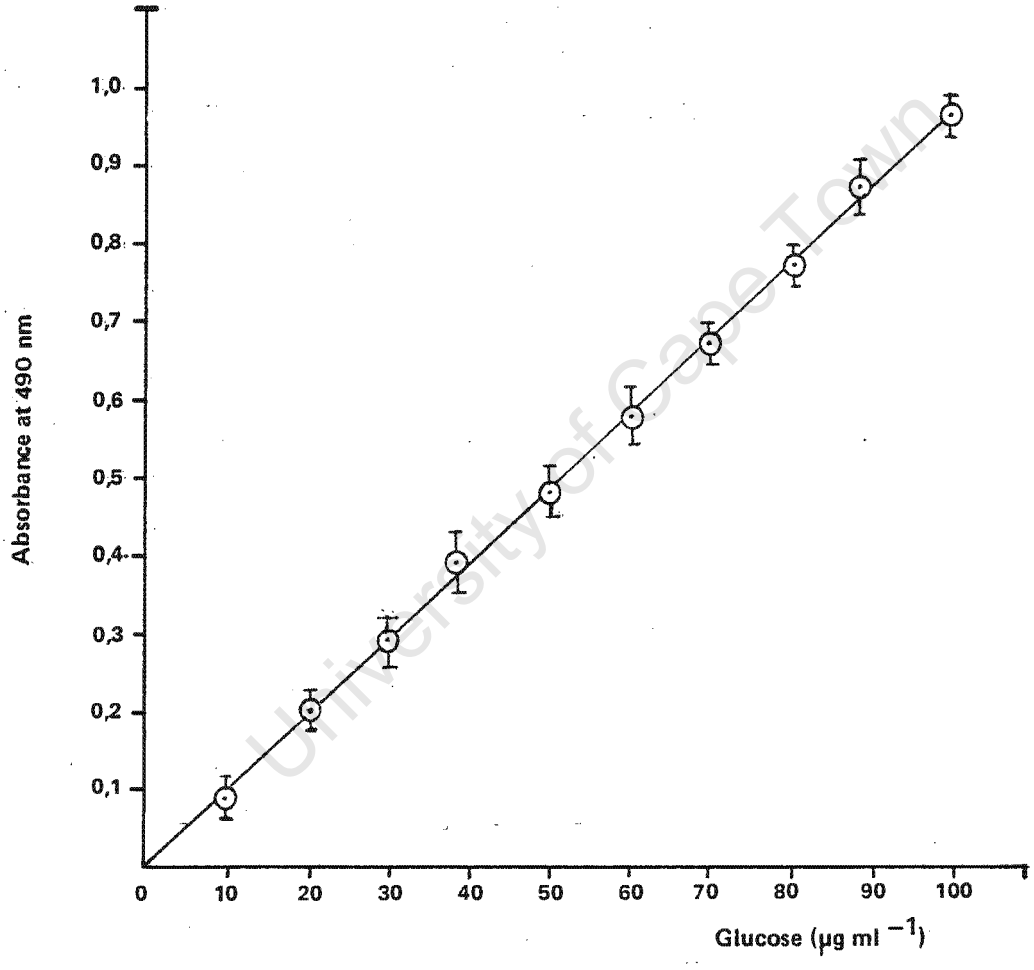


FIG. 9 A calibration curve for glucose used in the analysis of total sugars by the phenol sulphuric acid method (Dubois *et al.*, 1956)

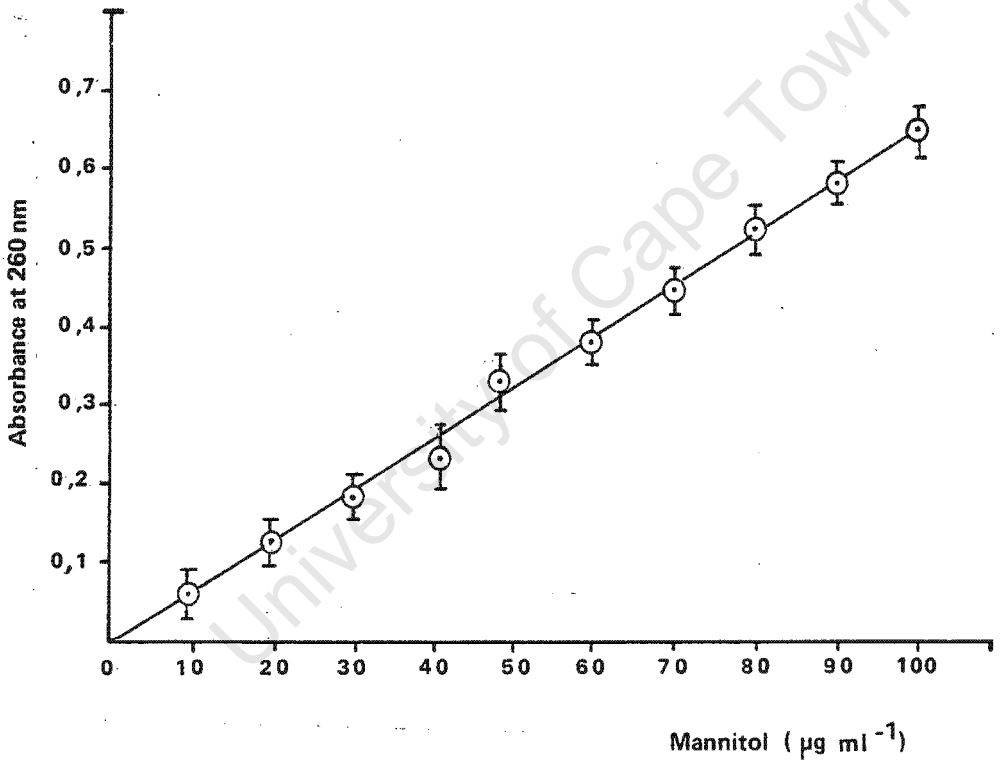


FIG.10 A calibration curve of mannitol oxidation using the periodate oxidation method (Lewis and Smith, 1967b), after one minute measured at an absorbance of 260 nm.

by Jensen *et al.* (1955). Titrimetric and decarboxylation are the most commonly used methods, but isolation of the alginic acid free from contaminants is extremely complicated (Jensen *et al.*, 1955). Colorimetric methods are useful if very small algin samples are present (this was not the case in *L. pallida* and *E. maxima*) and are general for uronic acids based on the work of Dische (1947)and not specific for alginic acid in the Phaeophyceae. The gravimetric method was chosen for this investigation as a quick and simple one. Since this study was carried out, Hay *et al.* (1983) have used colorimetric methods to determine the algin content of various brown algae in South-western Cape waters and found good correlation between gravimetric and colorimetric methods although the latter gave consistently lower results.

The ethanol-soluble carbohydrates were separated by paper and gas liquid chromatography. The paper chromatograms were prepared by spotting on to Whatman's no. 1 chromatography paper (46 x 57cm) concentrated samples and standard carbohydrates made up in 80% ethanol and developed by means of descending chromatography using ethyl acetate:acetic acid: water (14:3:3) solvent mixture in Shandon 500 chromatography tanks. The chromatograms were run for 38-42 hours at 20-22°C. Carbohydrate spots were detected by means of the silver nitrate/ sodium ethoxide method (Trevelyan *et al.*, 1950). After drying in a fume cupboard the paper was cut into longitudinal strips, dipped in a saturated solution of silver nitrate in acetone, dried and dipped in sodium hydroxide in 95% ethanol and again dried. Dark brown spots formed on a light brown background which was cleared by "fixing" in 10% sodium thiosulphate and washed in running water for thirty minutes.

It was found that the solvent ethyl acetate:acetic acid:water (14:3:3:)did not separate mannitol, sorbitol or dulcitol and another solvent, ethyl-methyl-ketone:acetic acid:water saturated with boric acid (9:3:1)was therefore used. Lewis and Smith (1967b) tabulated the mobilities of various sugars and polyols using this solvent. The components were detected using the silver nitrate spray of Trevelyan *et al.* (1950).

As a further check, another detection agent, modified

p-anisidine hydrochloride spray, was used to detect ethanol-soluble sugars and uronic acids. The original p-anisidine reagent of Hough *et al.* (1950) was acidified by the addition of 2mℓ concentrated HCl 100mℓ^{-1} (Lewis *et al.*, 1972). The dipped chromatograms were heated at 60° for five to ten minutes. Aldohexoses yielded a green/brown colour, keto-hexoses a lemon yellow colour, methylaldopentoses an emerald green colour and uronic acids a cherry red colour. All colours were enhanced under ultra violet light. Glucose was used as a marker on all chromatograms to allow the Rg values to be calculated from the following formula:

$$Rg = \frac{\text{Distance:Starting point - Centre of spot of "unknown" sugar}}{\text{Distance:Starting point - Centre of spot of glucose}}$$

Chromatograms of the ethanol-soluble fraction taken from ^{14}C-labelled plants had the glucose standard spot removed from the chromatogram and developed by the silver nitrate/sodium ethoxide method (Trevelyan *et al.*, 1950) as previously described. Autoradiographs were prepared by placing the chromatogram against Kodak X-ray film and incubating for six days in the dark. The X-ray film was then developed in Kodak DX-80 developer (diluted 1:4) for four minutes at 20°C and fixed in Kodak FX-40 X-ray liquid fix (diluted 1:4) for six minutes using two fixing baths. The film was fixed in the first bath until clear and then fixed for the same length of time in a second bath. The X-ray film was subsequently washed for thirty minutes before drying.

In the gas liquid chromatography investigations of carbohydrates a Pye-Unicam Series 104 Dual Column Analytical Gas Chromatogram with flame ionization detectors and glass columns (each 2,1m long x 6mm internal diameter) was used in conjunction with a Phillips PM 8 000 recorder. The recorder chart speed was set at 5mm min^{-1} and oxygen-free nitrogen 30mℓ min^{-1} was employed as a carrier gas. The column was packed with diatomite CQ as the solid support and 2% SE 52 (methyl-phenyl silicon gum) as the non-polar liquid phase. Samples of the ethanol-soluble fraction were deionized and deproteinized as previously described in section 2.3. Samples of algae were refluxed for twelve hours in 2N

trifluoroacetic acid to obtain the acid-hydrolysed fraction; 1mg $m\ell^{-1}$ erythritol was added to each sample as an internal standard to ensure correct detector response and carrier gas flow rate. The samples were prepared so as to yield the trimethylsilylate (TMS) derivatives of the carbohydrates. Each sample was freeze dried on a New Brunswick freeze drier in a 50mℓ pear shaped quick fit flask. Trimethylsilylate derivatives were prepared by redissolving the freeze dried sample in 0,85mℓ pyridine and adding, within five minutes, 0,1mℓ hexamethyldisilazane (HMDS) and 0,05mℓ trimethylchlorosilane (TMCS) to produce a reaction mixture of 1,0mℓ (Sweeley *et al.*, 1963). After vigorous agitation on a mechanical shaker for one minute the stoppered flasks were allowed to stand overnight before chromatography. Any coagulation of the fine white precipitate usually indicated the presence of water and these were discarded. A 2-10 $\mu\ell$ sample of the solution was injected into the instrument by means of a 10 $\mu\ell$ Hamilton syringe with a 4,5cm needle. The temperature programme was set at 140°C for four minutes, then at 140°C to 290°C with an increase of 6°C min^{-1} with a final isothermal temperature of 290°C for twenty minutes. Standards (1mg $m\ell^{-1}$) were run individually and in different combinations to aid identification of the components of the ethanol-soluble and acid-hydrolysed fraction. All standard sugars were placed in a dessicator prior to preparation of the trimethylsilylate derivatives. A chromatogram of standard carbohydrates and calibration curves for the same sugars are shown in Figs. 11 and 12 respectively. A quantitative estimate for each sugar could be made by measuring the height of each peak and referring to the calibration curves of standard carbohydrates (Holligan and Drew, 1971). Results of the hydrolysed polysaccharides are presented as mol% and galactose and glucose were estimated using the single completely resolved anomer peaks, namely α-galactose and β-glucose. The mol% of each component could be calculated from the following equation:

$$\text{Molar proportion} = \frac{\text{Peak height (or area) of carbohydrate}}{\text{molecular wt} \times K}$$

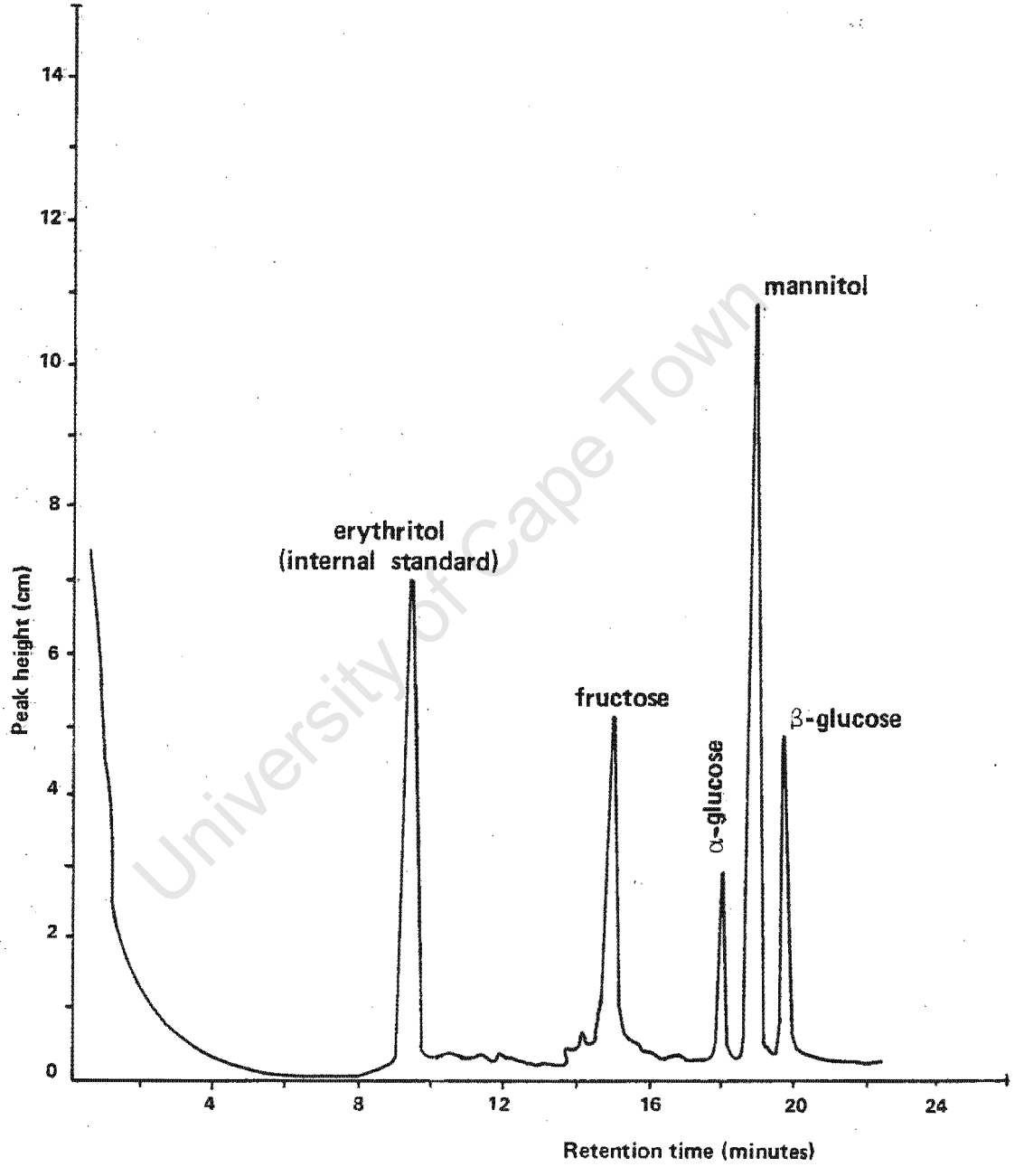


FIG.11 GLC chromatograms of various standard sugars

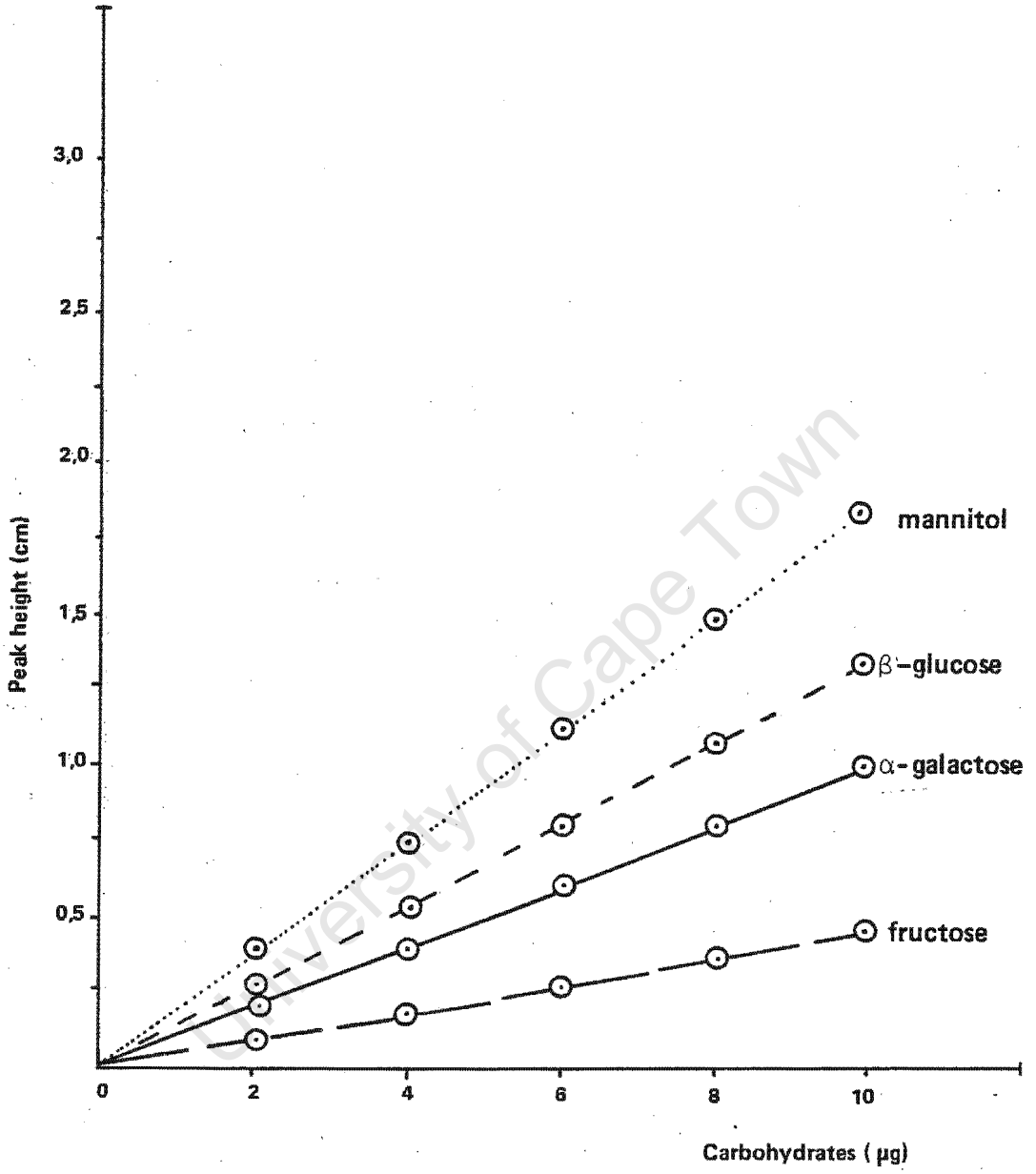


FIG.12 Calibration curves for TMS derivatives of standard carbohydrates

where K is a constant for the respective carbohydrates derived from the gas liquid chromatography calibration curves such that:

$$K = \frac{\text{peak height (or area) of carbohydrate/weight of carbohydrate}}{\text{peak height (or area) of internal standard/weight of internal standard}}$$

Analytical determination of soluble amino compounds were carried out using 200-500 µℓ of extract and a Beckman Model 120C Amino Acid Analyser. Acidic and neutral amino acids were separated on a 69cm column of Beckman PA35 spherical ion exchange resin using two lithium citrate buffers sequentially. The first buffer was 0,03 M lithium; 0,05 M citrate at a pH adjusted to 2,72 (Atkin and Ferdinand, 1970) and the second 0,03 M lithium; 0,21 M citrate at a pH adjusted to 3,73 (Kedenburg, 1971). A lithium citrate process was used as this facilitated the separation of the amides, asparagine and glutamine. Basic amino acids were separated on a short column of 12cm of Beckman PA 35 resin using a 0,35 M sodium buffer at pH 5,83 (Atkin and Ferdinand, 1970; Kedenburg, 1971). The amino acids, once separated, were combined with a ninhydrin solution, incubated at 100°C and the optical densities ascertained by means of a Honeywell Electronik 16 logarithmic recorder and a Beckman 125 digital integrator. The pool size in µmol $m\ell^{-1}$ was calculated using the digital integrator readings corrected by specific conversion constants for each amino acid. The conversion constants were determined from calibration runs. The major acidic and neutral amino acids were separated and collected from a 150 x 1,8cm Beckman M 84 ion exchange resin column. The eluted stream was split in the ratio of 1:10 (analytical system:collection system) using a stream divider system. Ammonium ions were separated on the short column. The eluate was automatically collected in both cases in test tubes by a LKB Ultrorac 7000 fraction collector. To determine which tubes contained the amino acids 200 µℓ from each tube was sequentially spotted onto Whatman's no. 41 filter paper and sprayed with ninhydrin. Incubation in an oven at 110°C for fifteen minutes developed the colour and a visual comparison with the peak printout from the recorder located the amino

acids.

In all the ^{15}N experiments portions of frond 10cm in length were excised under water and placed in beakers of seawater in a Conviron Model E15 growth chamber set at a constant temperature of 10°C, 93μE $m^{-2}s^{-1}$ and 80% humidity, and left for two hours to equilibrate to the environmental conditions prior to the addition of the isotope. The isotope used was ^{15}N-labelled potassium nitrate (99 atom percent ^{15}N, Prochem, London, U.K.). Two solutions, one of 25 μg $m\ell^{-1}$ and the other of 50 μg $m\ell^{-1}$ ^{15}N potassium nitrate, were initially used for an infiltration time of five, ten and sixty minutes, but at these isotope activities there was insufficient enrichment to determine which amino acids carried the ^{15}N-label. Another method of achieving higher enrichment values was to increase not only the concentration of the isotope solution but also to lengthen the time of infiltration. Plants were therefore incubated for two, four and six hours with two concentrations (200 μg $m\ell^{-1}$ and 400 μg $m\ell^{-1}$) of ^{15}N potassium nitrate.

All environmental conditions and times of infiltration were kept constant for the inhibitor experiments except that 5mM L-methionine DL-sulphoximine (MSO) (Sigma, London) was added to the ^{15}N-solution. After incubation, the metabolic processes were instantly stopped by plunging the plant material into liquid nitrogen, after which the plant material was homogenised in cold 80% ethanol (1g tissue 50$m\ell^{-1}$ ethanol) in an Ultra Turrax homogeniser. Extraction of the soluble amino compounds followed at 0°C for twenty four hours, the homogenate was filtered through Whatman no. 1 filter paper and then the ethanol extract evaporated under an airstream to a final volume of 10$m\ell$. Chlorophyll and lipid material were removed by shaking the sample with 5$m\ell$ of petroleum ether, freezing and pouring off the unfrozen petroleum ether.

The samples containing the amino acids were concentrated down to 10$m\ell$ and converted to ammonium sulphate by the Kjeldahl method. The sample was placed in a 50$m\ell$ micro-Kjeldahl flask, one BDH mercury catalyst tablet (containing

the equivalent of 0,1g mercury and 1,0g sodium sulphate) and 3mℓ of nitrogen free concentrated sulphuric acid was added. Sample digestion took two hours.

Digested samples were dissolved in 10mℓ of ammonia free distilled water and the ammonia distilled off in a Markham micro-distillation still after alkalisation with 15mℓ of 50% NaOH and 2,5% sodium thiosulphate. The sodium thiosulphate prevented the ammonia from binding with the mercuric oxide which occurred occasionally with the addition of sodium hydroxide. The distilled ammonia was collected in 2mℓ of 0,02N HCl and titrated against standardised 0,005N NaOH using screened methyl-red as an indicator. An optimum amount of 30 µg ammonium-N was required for each determination in the ^{15}N analytical system used. Subsequently, samples were acidified with 0,5mℓ of 0,1N HCl to prevent ammonia loss and concentrated down to a level at which 0,2mℓ contained 30 µg of ammonium-N.

The Faust (1967) method was used to prepare the discharge tubes. Sodium hypo-bromite, the oxidant, reacted with the ammonia under vacuum to release nitrogen; the sequence is as follows (Kamen, 1957):

$$2NH_3 + 3NaOBr \longrightarrow N_2 + 3H_2O + 3NaBr$$

This reaction took place under a vacuum of 0,1 Pa achieved by using a mercury diffusion pump backed up by an Edwards rotary high vacuum pump. The vapour pressure in the vacuum system was further reduced by the presence of liquid nitrogen cold traps. The nitrogen resulting from this reaction was sealed in discharge tubes and analysed in a Packard N-15 Statron NOI-4 atomic emission spectrophotometer. A high frequency discharge in the equipment excited the nitrogen gas in the discharge tube which emitted a violet light. In those cases where a blue colour resulted the tube was discarded as this indicated contamination either by water vapour or bromine. The instrument photoelectrically recorded intensity of the band heads for the three molecules of nitrogen; $^{14}N^{14}N$ at 315,0nm; $^{14}N^{15}N$ at 316,2nm and $^{15}N^{15}N$ at 316,6nm. An example of a typical trace is shown in Fig. 13.

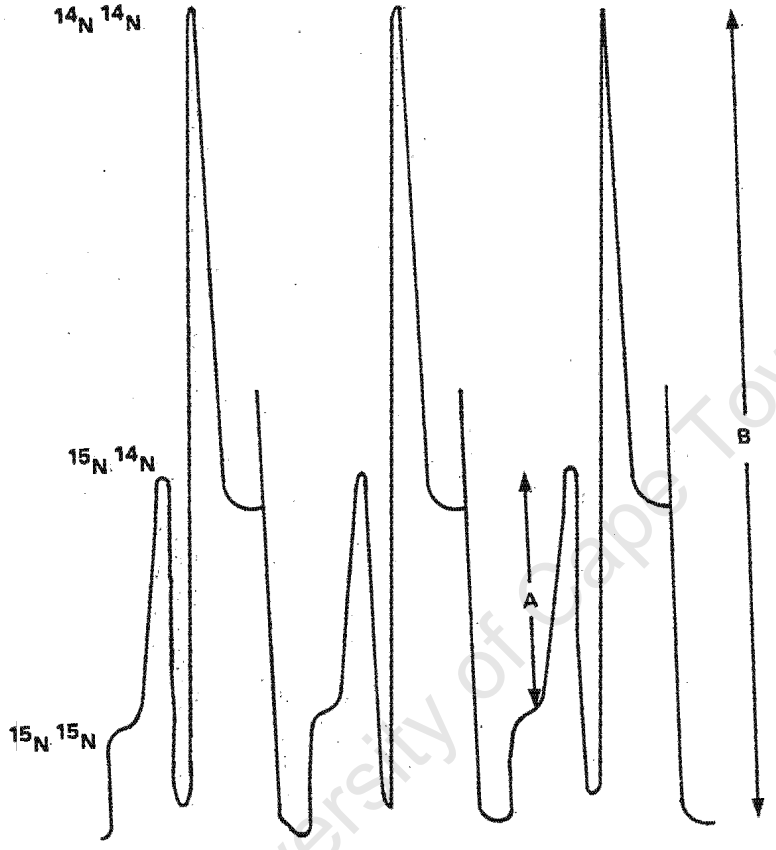


FIG.13 Typical traces showing good separation of the three hybrid molecules of nitrogen $^{14}N^{14}N$; $^{15}N^{14}N$; $^{15}N^{15}N$. A shows the peak height of the $^{15}N^{14}N$ bandhead while B shows the peak height of the $^{14}N^{14}N$ bandhead.

The enrichment was calculated using the following formula:

$$En\% = \frac{100}{2(A/B \times Vb/Va) + 1}$$

where En% is the percentage enrichment, A is the bandhead of the $^{14}N^{14}N$ molecule, B the bandhead of the $^{15}N^{14}N$ molecule and Va and Vb the gain settings of the atomic emission spectrophotometer at which the bandheads A and B are recorded.

All ^{15}N enrichment results were corrected using a standard curve derived from a set of standards provided by Packard Instruments. The final percentage enrichment in excess of the natural abundance (A%E) was obtained by subtracting the natural abundance of 0,37% from the corrected percentage enrichment. The ^{15}N content of a particular amino pool, expressed as $\mu g\ ^{15}N\ gfm^{-1}$ (gram fresh mass) was determined by multiplying the pool size of the amino acid by its ^{15}N enrichment and the number of nitrogen atoms per molecule of the amino compound.

2.4 ^{14}C-labelling Studies using isolated *C. minima* and *S. vittata* attached to their respective algal stipes

The ^{14}C-studies on *C. minima* and *S. vittata* involved labelling with two isotopes, $Na_2H^{14}CO_2$ and ^{14}C-mannitol. In the experiments using ^{14}C-bicarbonate, sections of stipe, about 14cm in length, of *L. pallida* with attached *C. minima* and *E. maxima* with attached *S. vittata* were immersed in filtered seawater and left for two hours to equilibrate. Sections of stipe were chosen which possessed a very dense covering of red algae so that the only portions of stipe exposed to direct light were the two cut ends. The ^{14}C-bicarbonate was added ($1\mu Ci\ m\ell^{-1}$) and left for the sixteen hours of the experiment. Samples of red algae (six replicates per sample) were taken every two hours and washed in three changes of filtered seawater to remove any residual isotope. The whole plant was weighed and oven-dried at 65°C overnight. The oven-dried samples were weighed, solubilized, using the method of Lobban (1974) and then 10mℓ Dimilume-30 was added. The activity was counted and converted to DPM g^{-1} dry mass (see section 2.3).

In the experiments to determine whether *C. minima* and

S. vittata would utilize exogenously supplied mannitol, the red algae were carefully separated from the brown algal stipes, placed in small beakers of filtered seawater in a Conviron growth chamber at 10°C ± 0,5°C, 80% humidity and 93μE $m^{-2}s^{-1}$ at the plant surface. Plants were left for two hours to equilibrate, the isotope ^{14}C-mannitol (0,5μCi $m\ell^{-1}$) added and samples taken after four, eight and twenty four hours incubation. Samples were washed in filtered seawater to remove residual isotope and oven-dried at 65°C overnight. They were then fractionated using the method of Callow *et al.* (1979). The three fractions were an 80% ethanol-soluble fraction, a starch fraction obtained by hydrolysis in α-amylase and an acid fraction obtained by 90% formic acid extraction. In all cases the soluble and insoluble portions were separated by centrifugation, the supernatants evaporated to dryness, blended with Beckman Ready Solv™GP premixed liquid scintilation cocktail and the activity monitored on a Beckman LS 150 liquid scintillation spectrophotometer. The counts were converted to disintegrations per minute (see section 2.3).

All other physiological studies on *C. minima* and *S. vittata* using paper chromatography, GLC and amino acid analysis, were identical to those described for brown algae in section 2.3.

The photosynthetic pigments of the red algae were extracted by grinding shredded algae, frozen in liquid nitrogen, in a cold pestle and mortar. Carotenes and chlorophylls were removed by the addition of 50mℓ of 90% acetone, which was then filtered into a separating funnel and 20ml of petroleum ether added. The pigments were contained in the petroleum ether and concentrated in a rotoevaporator. Water soluble pigments were extracted by the addition of 50mℓ distilled water to the frozen, ground algae and the mixture left in the dark for two hours and pigments concentrated in a roto-evaporator. All pigments were scanned on a Pye-Unicam SP 1800 ultra violet spectrophotometer from 390-700nm and identified using the absorption peaks of algae pigments of Govindjee and Braun (1974).

2.5 Physiology of the *L. pallida/C. minima* and *E. maxima/ S. vittata* associations

Plants of *L. pallida* with *C. minima* and *E. maxima* with *S. vittata* were placed in the perspex chambers (Fig. 8). The fronds were exposed to ^{14}C-labelled seawater but the stipes, with attached red algae, were placed in the lower chamber containing the non-radioactive filtered seawater. These experiments were carried out and analysed in an identical manner to that already described for kelp plants without attached red algae (see section 2.3). Only red algae attached to the stipe which had not directly been exposed to ^{14}C-label were analysed. Experiments were carried out using red algae attached to portions of brown algal stipe which were incubated in 1 µCi $m\ell^{-1}$ $NaH^{14}CO_3$ in seawater to show if the red algae attached to brown algae could incorporate ^{14}C-label by photosynthesis. Paper chromatograms of the ethanol-soluble fraction from plants of the above experiment were run and analysed as described in section 2.3 for the brown algae without attached red algae. Autoradiographs were prepared from the chromatograms and gas liquid chromatography studies and amino acid analyses were carried out in the same manner as for brown algae without attached red algae as described in section 2.3.

In the anatomical studies 20 µm sections of the stipe of brown algae with attached red algae were cut on a Pelcool freezing microtome and examined under a Zeiss Photomicroscope at magnifications of 25 to 100 times to investigate the morphological differences between *L. pallida* and *E. maxima* and the degree of penetration by the red algae.

CHAPTER 3

THE PHYSIOLOGY AND ANATOMY OF *LAMINARIA PALLIDA* and *ECKLONIA MAXIMA*

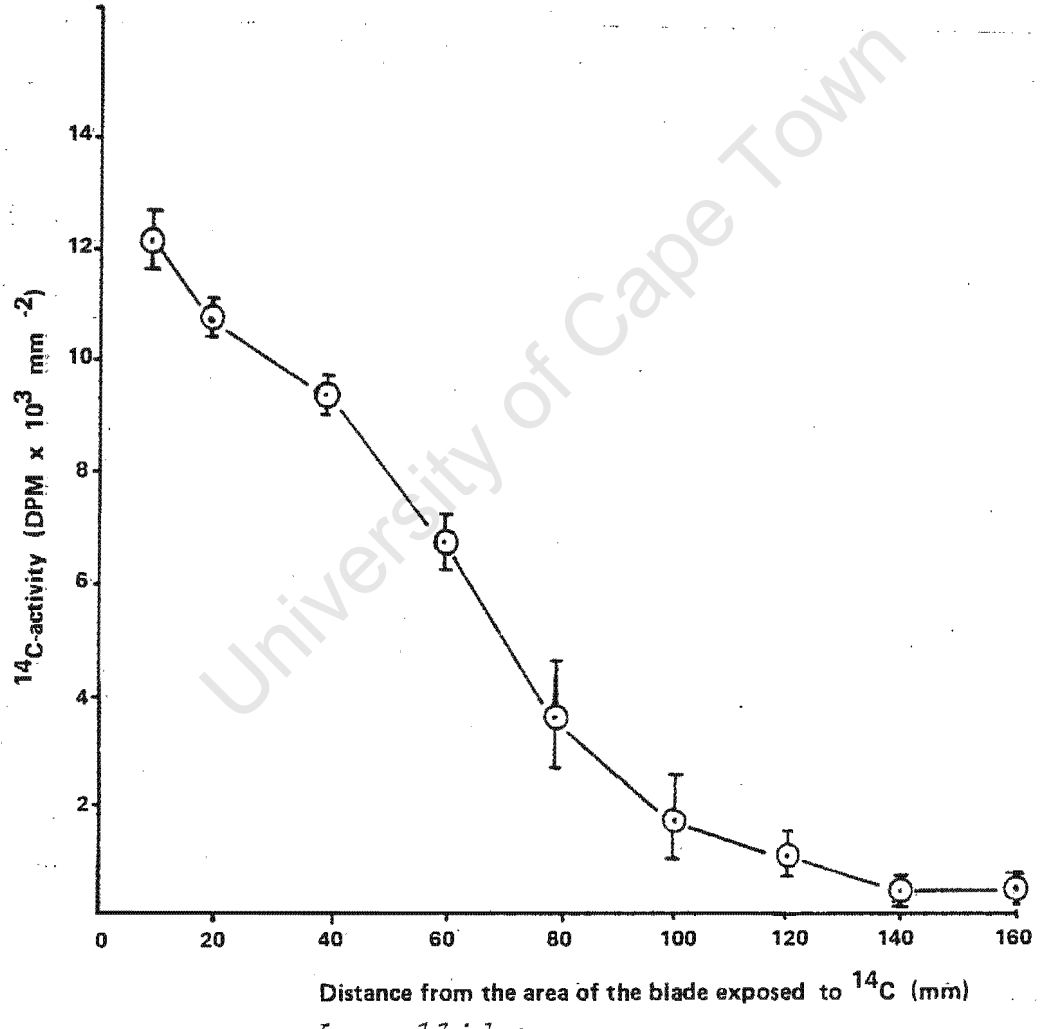
3.1 Introduction

The overall aim of this investigation was to study the *L. pallida/C. minima* and *E. maxima/S. vittata* associations, but it became apparent that, due to a lack of data on the physiology and biochemistry of these algae, it was necessary to investigate both the brown and red algae separately before attempting any study on their relationships. This chapter covers the aspects of brown algal physiology and anatomy which would aid in an understanding of the brown/red algal relationship. The areas covered include the velocities of translocation in the brown algae, the major products of translocation after ^{14}C-labelling experiments and variation in the carbohydrates and amino acids of the algae.

3.2 Velocity of Translocation of ^{14}C-labelled Assimilates

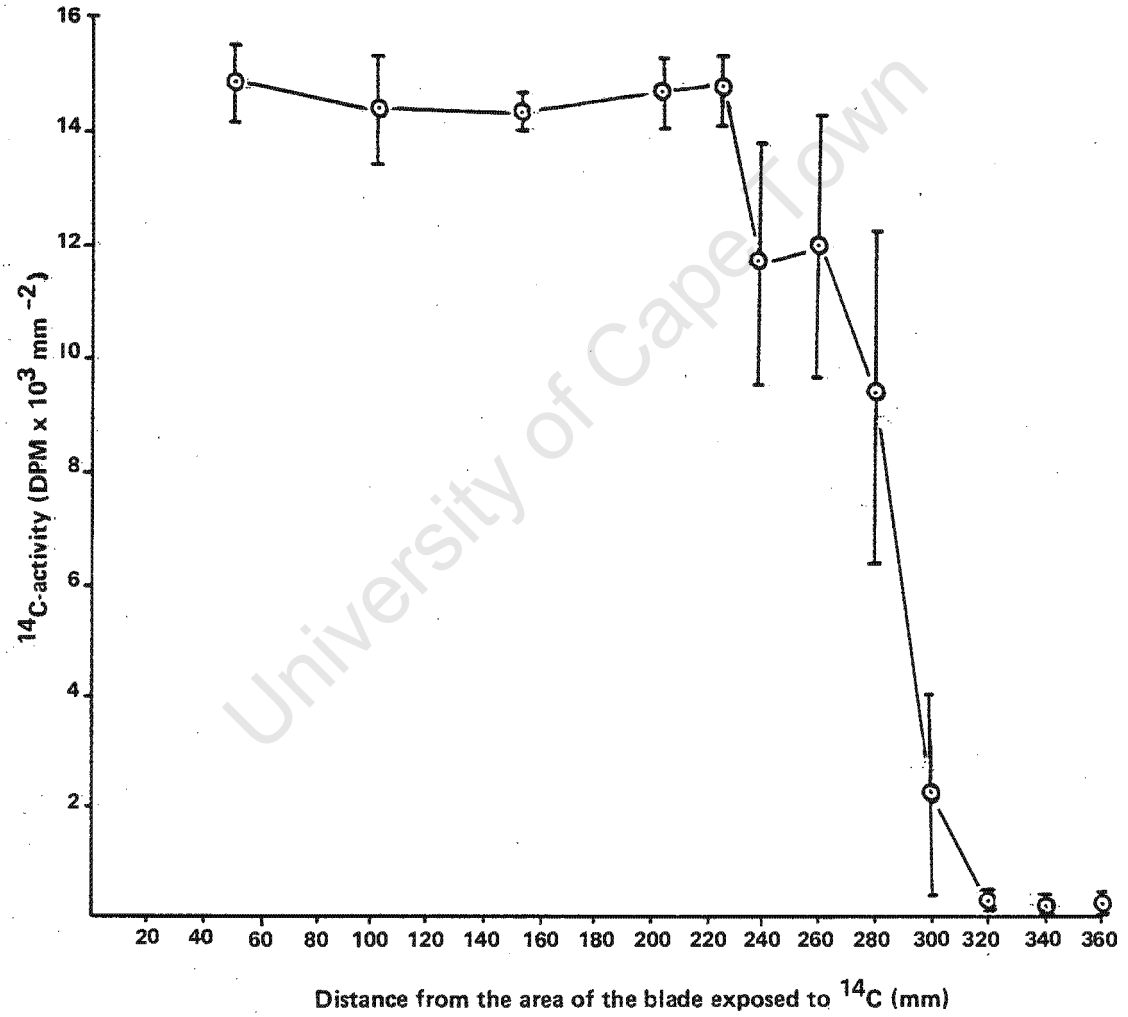
A preliminary study on translocation was undertaken using a perspex chamber filled with ^{14}C-isotope which was glued to the frond. This experiment showed that after two hours incubation the front of radioactivity moved 163mm in *L. pallida* and 507mm in *E. maxima* which indicated a velocity of 82mm h^{-1} in *L. pallida* and 254mm h^{-1} in *E. maxima*.

The results of more detailed experiments using six plants are shown in Fig. 14 for *L. pallida* and Fig. 15 for *E. maxima*. (The data from which these figures were plotted are presented in Tables 1 and 2 of the Appendix). The velocity of movement was faster in *E. maxima* than in *L. pallida* and in the latter (Fig. 14) the average rate of movement of ^{14}C-labelled assimilates between the six plants gradually decreased with distance travelled from the site of exposure to the ^{14}C-label suggesting a passive movement along the frond. All the plants of *E. maxima* (Fig. 15) seemed to translocate the



L. pallida:

FIG 14 The distribution of ^{14}C-labelled assimilates along the lamina after one hour photosynthesis in ^{14}C-labelled seawater. Vertical bars represent twice the SEM and each point is a mean of four replicates from each of six plants.



E. maxima:

FIG. 15 The distribution of ^{14}C-labelled assimilates along the lamina after one hour photosynthesis in ^{14}C-labelled seawater. Vertical bars represent twice the SEM and each point is a mean of four replicates from each of six plants.

^{14}C-labelled assimilates to approximately the same distance from the site of attachment of the perspex ampoule (i.e., about 220mm) and then a sharp decrease occurred in the amount of ^{14}C-label present (Fig. 15). Beyond 220mm the six plants of *E. maxima* used in the experiment continued to transport the ^{14}C-labelled assimilates for different distances from the site of attachment of the ampoule, the range being from 240-320mm. There was no radioactivity present in the samples at a distance of over 320mm from the site of exposure to the ^{14}C-label. These results indicate that the velocity of movement in *E. maxima* was between 240 and 300mm h^{-1} which corresponds to the preliminary results of 254mm h^{-1}. The velocity of movement in *L. pallida* was between 50 and 100mm h^{-1} (Fig. 14) which corresponds to 82mm h^{-1} found in the preliminary studies. This velocity in *L. pallida* is consistent with published data, summarized in Table 9. The small pore size of the Laminariales, 0,05µm diameter in *Sacchorhiza dermatodea* Batt. (Emerson *et al.*, 1982) and 0,06-0,09µm in *L. saccharina* (Sideman and Scheirer, 1977) are responsible for the slow transport in the Laminariales. *Sacchorhiza dermatodea* has an anatomy differing from other Laminariales (Emerson *et al*, 1982).

A microscopic examination of sections of the central portion of the sporophyte of *L. pallida* and *E. maxima* revealed that *L. pallida* possessed sieve elements which greatly elongated, although their diameter at the cross walls remained the same resulting in a trumpet shape (Pl. 2A). There was a complex network of sieve elements and hyphal cells running in all directions but no signs of cross connections or branching (Pl. 2B). *Ecklonia maxima* had sieve elements which were larger in diameter than *L. pallida* (20µm and 14µm respectively) and showed distinct connections between sieve elements (Pl. 3A and 3B) but did not exhibit the distinctive trumpet shape present in *L. pallida*. The innermost cortical cells continually produce additional sieve elements which are superimposed upon each other to form "sieve tubes" running mainly longitudinally in the thallus, but also running radially as in *Alaria* (Schmitz and Srivastava, 1975). The complex network resulting from the superimposition of sieve elements is present in *L. pallida* (Pl. 2B) and *E. maxima* (Pl. 3A and 3B) and eventually older sieve elements become stretched and non-funtional.

PLATE 2

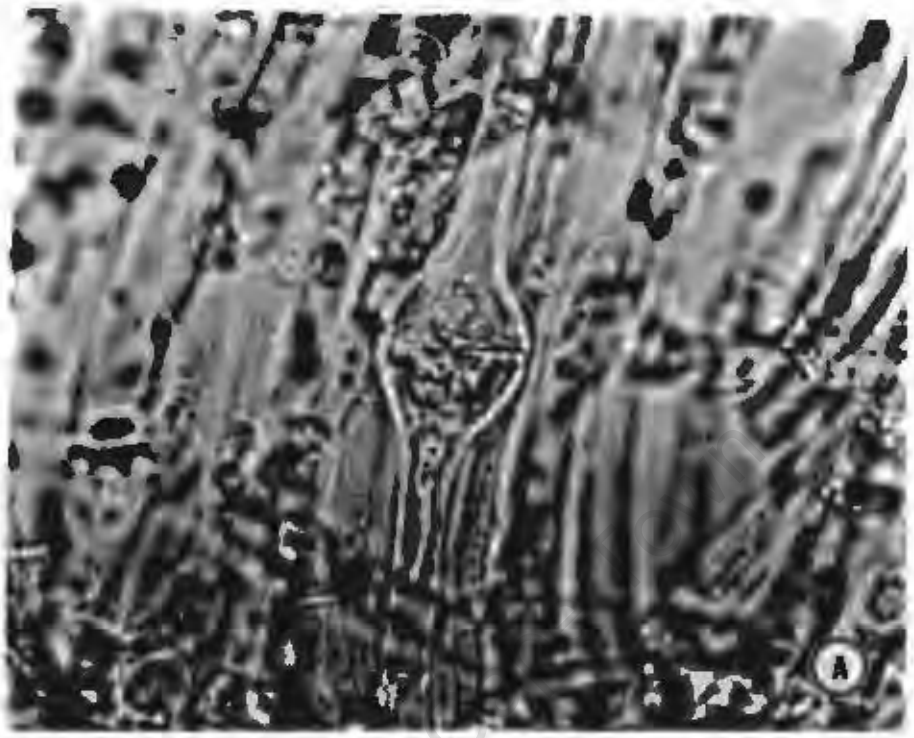
A

A section through the medulla of *L. pallida* showing a sieve element, in longitudinal section, which has greatly elongated, although the diameter at the cross wall remained the same resulting in a trumpet shape

B

A section through the medulla of *L. pallida* showing the complex network of sieve elements and hyphal cells running in all directions but with no sign of cross-connections between sieve elements

PLATE 2



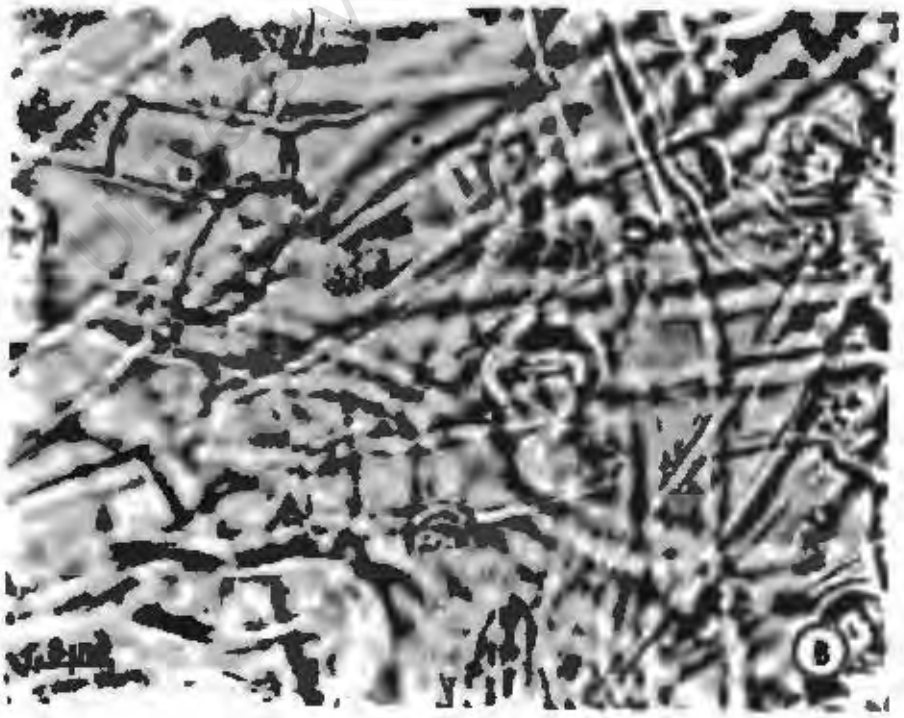


PLATE 3

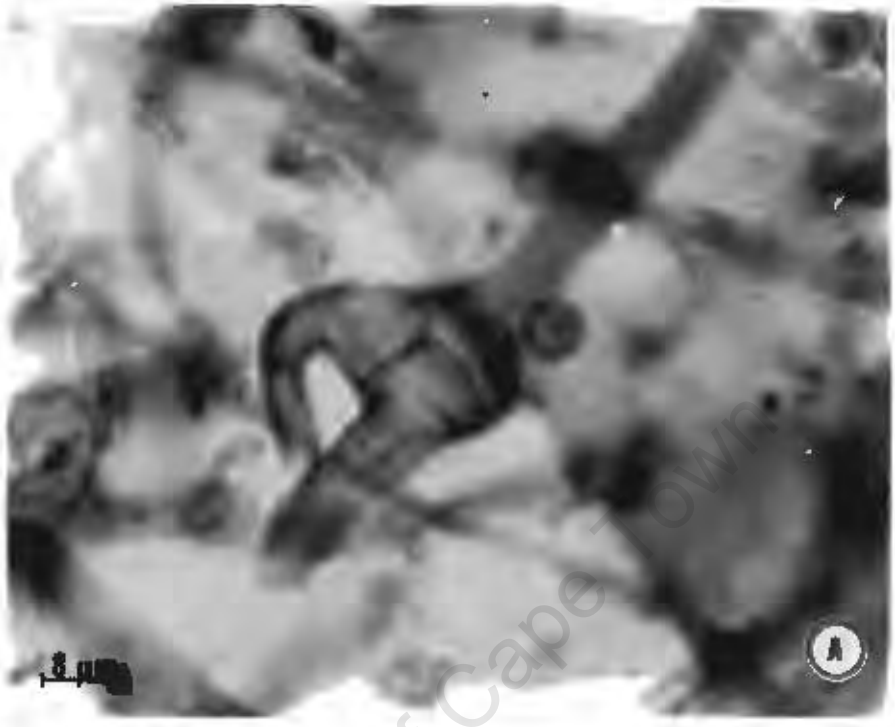
A

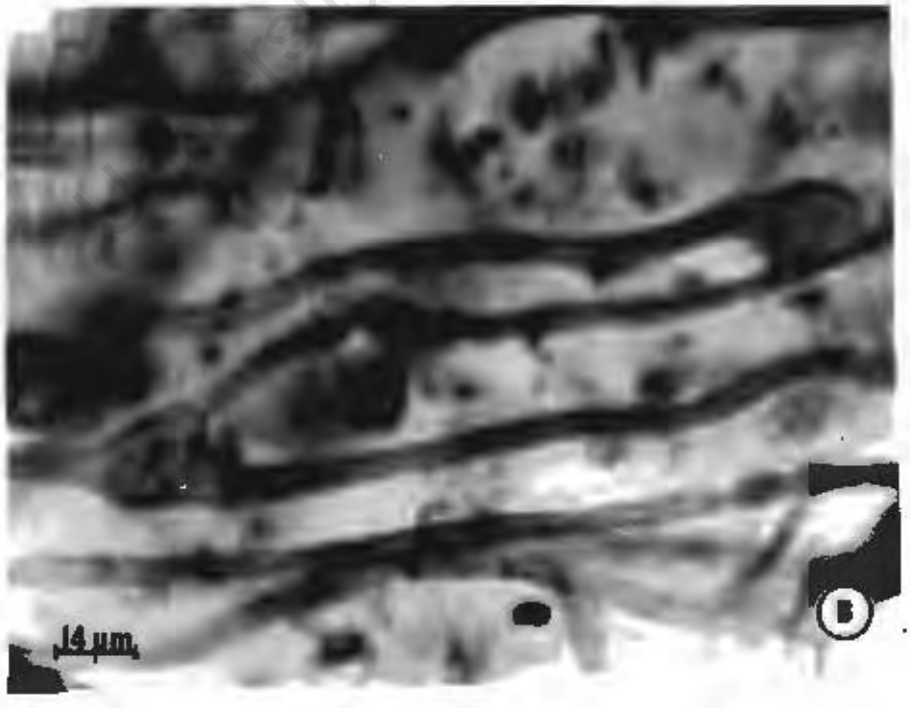
A section through the medulla of *Ecklonia maxima* showing in detail the distinct connections found between the sieve elements

B

A section through the medulla of *Ecklonia maxima* showing how two different sieve elements are joined to form a branched network. The sieve elements do not show the trumpet shape present in *L. pallida*

PLATE 3





3.3 Translocation of ^{14}C-labelled Assimilates from Frond to Stipe

The results, expressed as percentage exported and percentage present in each region for *E. maxima* and *L. pallida*, are given in Table 1. In *E. maxima* a greater percentage of the ^{14}C-label incorporated by the frond and meristematic region was translocated to the stipe and holdfast than in *L. pallida* (Table 1). In both these laminarian algae the majority of the translocated ^{14}C-label was present in the stipe with only a small proportion in the holdfast (Table 1). A t-test showed that there was a significant difference at the 5% level in the percentage exported activity between these brown algae.

Other experiments were carried out to establish that movement of ^{14}C-labelled assimilates from frond to stipe was due to internal transport and not to some external leakage. The seawater in the bottom chamber (Fig. 8) which had a volume of 20ℓ yielded overall counts of less than 2 000 DPM in all experiments (i.e., less than 20 DPM mℓ^{-1}). It was found that untreated seawater gave counts of approximately half this figure due to a high salt content which reduced the counting efficiency. Plants of *L. pallida* and *E. maxima* were found to incorporate little ^{14}C-label in the frond if incubated in the dark (about 500 DPM g^{-1} dry mass for *L. pallida* and 700 DPM g^{-1} dry mass for *E. maxima* after six hours exposure to ^{14}C-label). In experiments carried out in the light the time of the experiment was important as plants of *L. pallida* exposed to $NaH^{14}CO_3$ in seawater for the first six hours of the light period resulted in an average count of 51 537 DPM g^{-1} dry mass from frond tissue. Plants exposed for the last six hours of the light cycle resulted in an average count of 33 517 DPM g^{-1} dry mass from frond tissue despite identical plant sizes, environmental conditions and length of incubation in the ^{14}C-label. Incubation in ^{14}C-isotope of all plants in this study began two hours after the start of the light cycle.

3.4 Identification of the Major Carbohydrates

Ethanol-soluble carbohydrates of the stipe and frond of

TABLE 1

The percentage distribution of recovered ^{14}C-label in *L. pallida* and *E. maxima* after six hours incorporation. Only the frond was incubated in the ^{14}C-bicarbonate ($1\mu Ci\ m\ell^{-1}$). Results are the means of seventy two replicates taken from twelve plants.

Region of brown algae	*L. pallida*	*E. maxima*
* Incubated Frond	86,1 (68,1 ± 2,3)	79,8 (63,3 ± 1,8)
* Stipe	12,1 (20,3 ± 1,0)	18,2 (25,3 ± 1,4)
* Holdfast	1,8 (7,7 ± 0,9)	2,0 (8,1 ± 1,0)
* Percent exported from frond	13,9 (21,9 ± 1,0)	20,2 (26,8 ± 1,7)
° Total activity	36,0 ± 2,9	49,5 ± 3,6

* Percent of total with angular transformation ± SEM in parentheses

° Total activity (DPM x 10^3 g^{-1} dry mass ± SEM)

L. pallida and *E. maxima* are shown in Table 2 and were obtained using the method of Dubois *et al.* (1956). The concentration of ethanol-soluble carbohydrates was slightly higher in *E. maxima* than in *L. pallida* and the stipe of both algae contained more carbohydrates than the frond. The frond is the major region of photosynthesis whereas these results indicate that the stipe is a region of carbohydrate storage. The ethanol-soluble fraction contained the majority of the ^{14}C-labelled assimilates when the plant was divided into various fractions, namely, ethanol-soluble, laminaran, alginic acid and residue (Table 3). Ethanol-soluble extracts from the brown algae were run on paper chromatograms. An example of a typical chromatogram showing the presence of the acyclic polyol mannitol is shown in Pl. 4A for *L. pallida* and Pl. 4B for *E. maxima*. Glucose was run on each chromatogram as a marker. The Rg value of mannitol was calculated from a standard to be 1,19 ± 0,001 SEM. An example of a typical autoradiograph developed from a paper chromatogram is shown for *L. pallida* in Pl. 5A and for *E. maxima* in Pl.5B. The TMS derivatives of the ethanol-soluble and the acid-hydrolysable fractions of the algae were analysed by gas liquid chromatography (GLC). A typical GLC trace of the ethanol-soluble fraction is shown in Fig. 16 for *Laminaria* and *Ecklonia*. The GLC traces for the acid hydrolysed fraction of *L. pallida* and *E. maxima* are shown in Fig. 17. The major component identified in the ethanol fractions of both algae was the acyclic polyol mannitol, while in the acid-hydrolysed fraction additional components were identified including glucose, mannose and galactose. The actual quantities of the components identified are given beside the GLC traces in Figs. 16 and 17.

3.5 Seasonal Variation in Mannitol

The concentration of the acyclic polyol mannitol in the frond, meristematic region and stipe of the two brown algae was measured at monthly intervals for eighteen months and the results for *L. pallida* and *E. maxima* are shown in Figs. 18 and 19 and Appendix Tables 3 and 4. Each monthly

TABLE 2

The total carbohydrates of a deproteinized and deionized ethanol-soluble fraction in *L. pallida* and *E. maxima* calculated as glucose units using the method of Dubois *et al.* (1956). Results are the mean of three samples taken every month from August to December 1979. There was no significant difference between the values obtained for each month

Algae		Total carbohydrates in mg g^{-1} dry mass ± SEM
L. pallida	Frond	74 ± 0,93
	Stipe	104 ± 1,31
E. maxima	Frond	86 ± 0,82
	Stipe	112 ± 0,96

TABLE 3

The percentage distribution of recovered ^{14}C-activity in the ethanol-soluble, alginic acid, laminaran and residue fraction of *L. pallida* and *E. maxima* after six hours incorporation. Only the frond was incubated in the ^{14}C-bicarbonate ($1\mu Ci\ m\ell^{-1}$). The residue fraction is what remains after extraction of the first three fractions and samples are the means of thirty six replicates taken from six plants.

Laminaria pallida

Region of Brown Algae	Ethanol-soluble	Alginic acid	Laminaran	Residue
*Incubated Frond	76,5 (61,0 ± 1,8)	1,3 (6,4 ± 0,6)	2,7 (9,5 ± 0,8)	5.2 (13,1 ± 0,7)
*Stipe i.e. percent exported	7,5 (15,9 ± 1,2)	1,1 (5,9 ± 0,6)	0,2 (2,3 ± 1,0)	5,5 (13,5 ± 0,9)
*Distribution	84,0 (66,4 ± 1,5)	2,4 (9,0 ± 0,8)	2,9 (9,9 ± 0,6)	10,7 (19,1 ± 1,0)
°Total activity	27,5 ± 1,6			

Ecklonia maxima

Region of Brown Algae	Ethanol-soluble	Alginic acid	Laminaran	Residue
*Incubated Frond	72,8 (58,6 ± 2,0)	1,5 (6,9 ± 0,6)	3,6 (11,0 ± 0,6)	7,1 (15,5 ± 0,9)
*Stipe i.e. percent exported	9,9 (18,3 ± 0,7)	1,7 (7,5 ± 0,8)	0,5 (4,0 ± 0,6)	2,9 (9,9 ± 1,0)
*Distribution	82,7 (65,4 ± 1,3)	3,2 (10,3 ± 0,7)	4,1 (11,7 ± 0,6)	10,0 (18,2 ± 0,8)
°Total activity	32,7 ± 1,9			

*Percent of total with angular transformation ± SEM in parentheses

°Total activity (DPM x 10^3 g^{-1} dry mass ± SEM)

PLATE 4

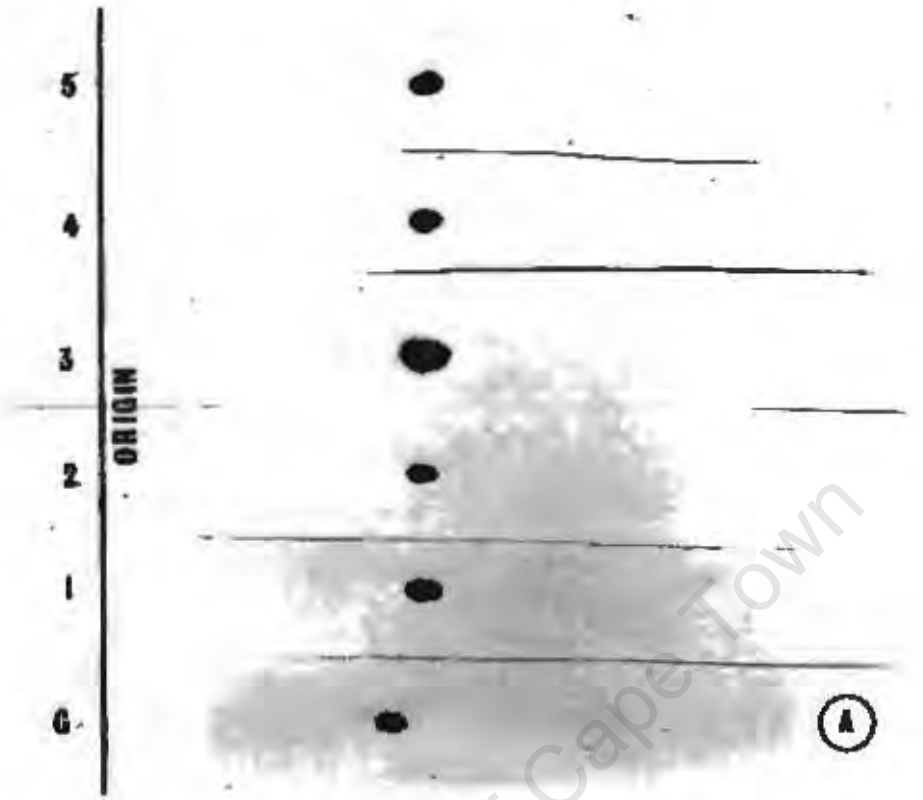
A

A paper chromatogram of an ethanol-soluble extract of the stipe of *L. pallida* run for forty hours at 20-22°C in ethyl acetate:acetic acid:water (14:3:3) by means of descending chromatography. Carbohydrate spots were detected by the silver nitrate/sodium ethoxide method of Trevelyan *et al.* (1950). The origin is marked, numbers 1-5 are replicates of *L. pallida* and G is the standard glucose marker

B

A paper chromatogram of an ethanol-soluble extract of the stipe of *E. maxima* run for forty hours at 20-22°C in ethyl acetate:acetic acid:water (14:3:3) by means of descending chromatography. Carbohydrate spots were detected by the silver nitrate/sodium ethoxide method of Trevelyan *et al.* (1950). The origin is marked, numbers 1-5 are replicates of *E. maxima* and G is the standard glucose marker

PLATE 4



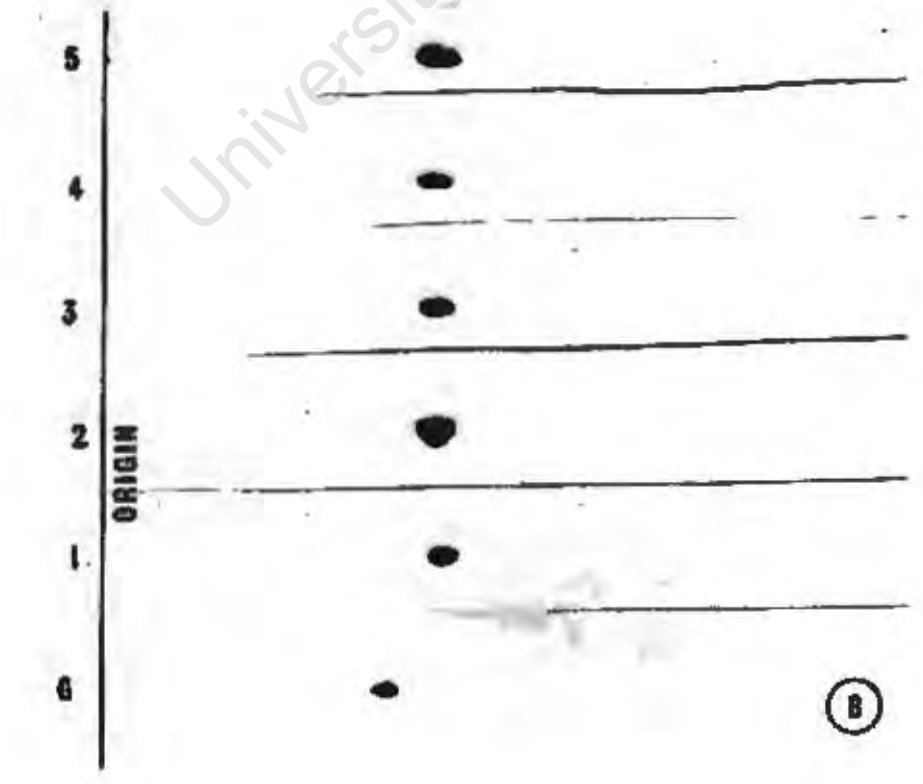


PLATE 5

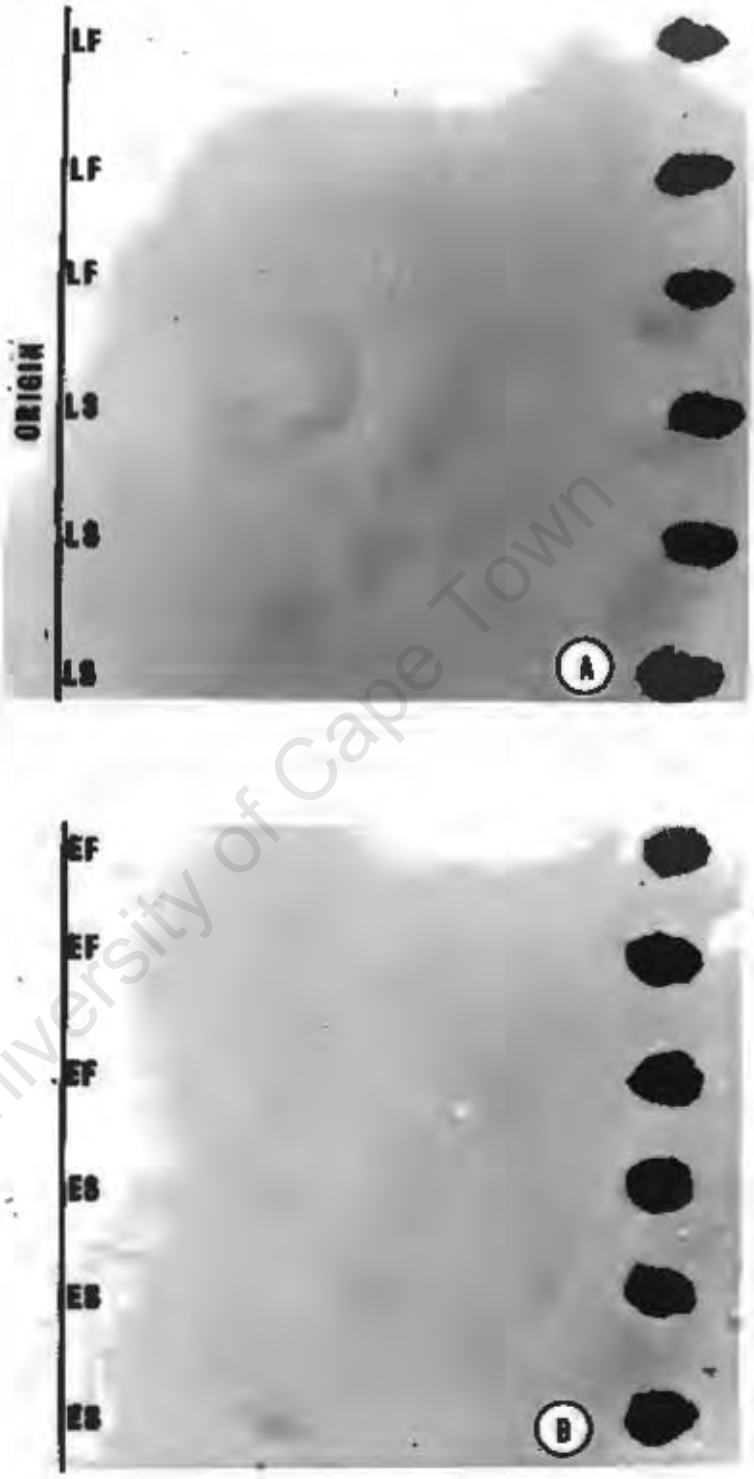
A

An autoradiograph on Kodak X-ray film prepared from a paper chromatogram, after six days exposure, of the ethanol-soluble fraction of the stipe (L.S.) and the frond (L.F.) of *L. pallida*

B

An autoradiograph on Kodak X-ray film prepared from a paper chromatogram of the ethanol-soluble fraction of the stipe (E.S.) and frond (E.F.) of *E. maxima*

PLATE 5



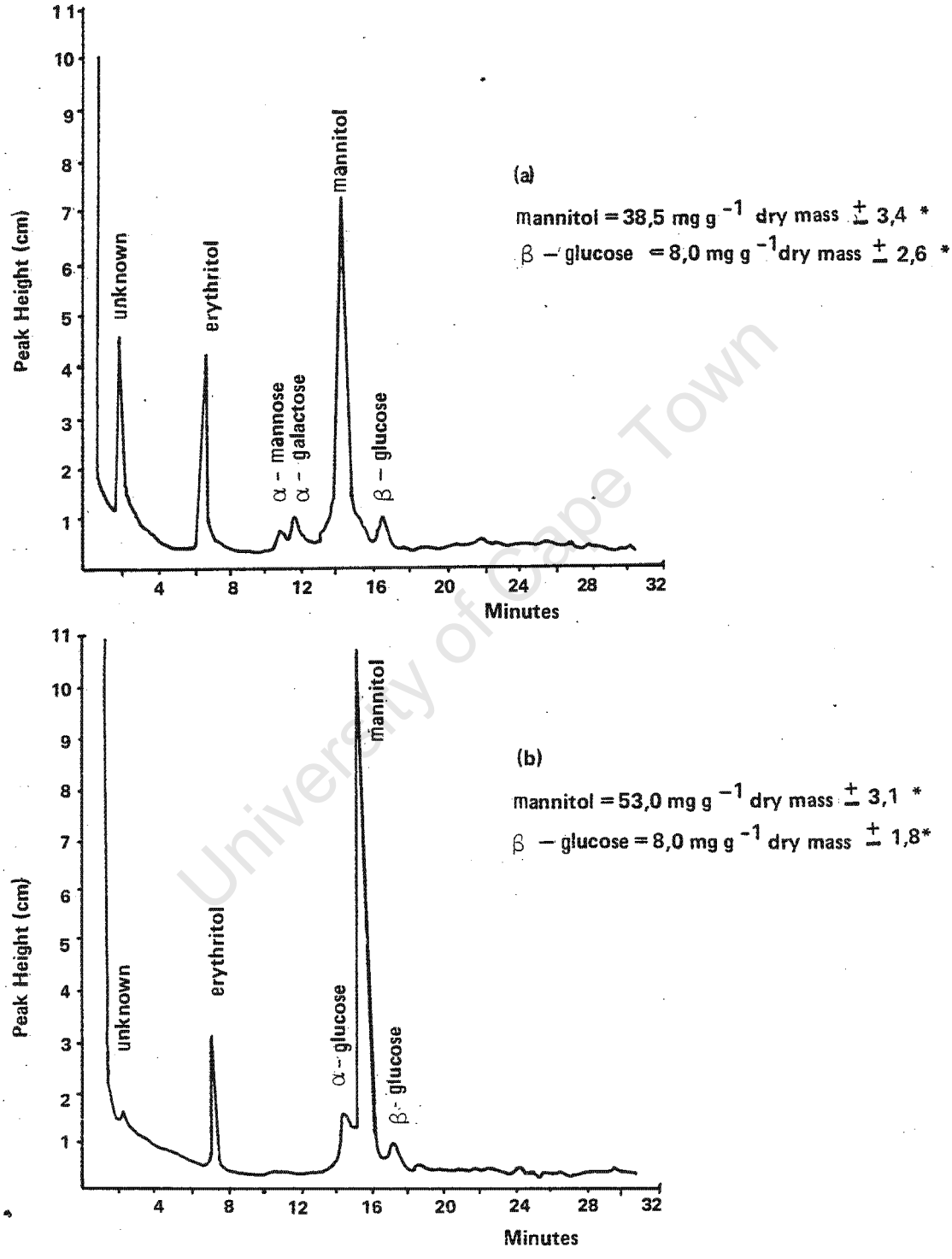


FIG.16 GLC chromatograms of the TMS derivatives of the ethanol-soluble fraction of the stipe of (a) *L. pallida* and (b) *E. maxima* Erythritol was used as an internal standard.
* Each is a mean of 3 values ± SEM.

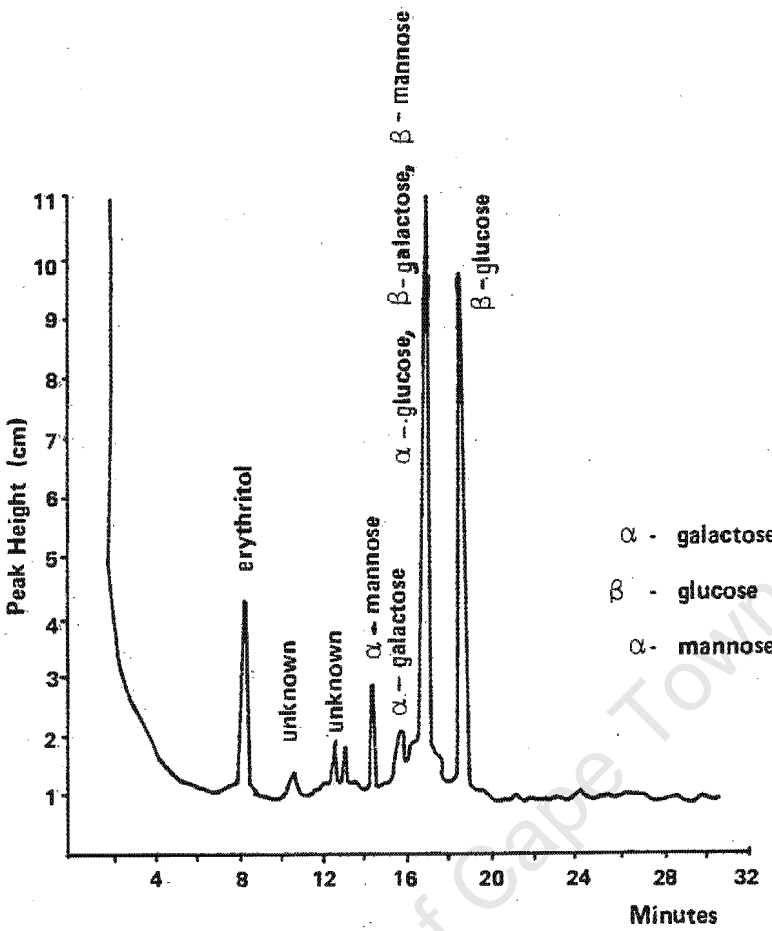


FIG.17 GLC chromatograms of the TMS derivatives of the acid-hydrolysed fraction of the stipe of (a) *L. pallida* and (b) *E. maxima*. Erythritol was used as an internal standard.
* Expressed as mol - % , angular transformations of percentages are shown in brackets ± SEM. Each is a mean of 3 values.

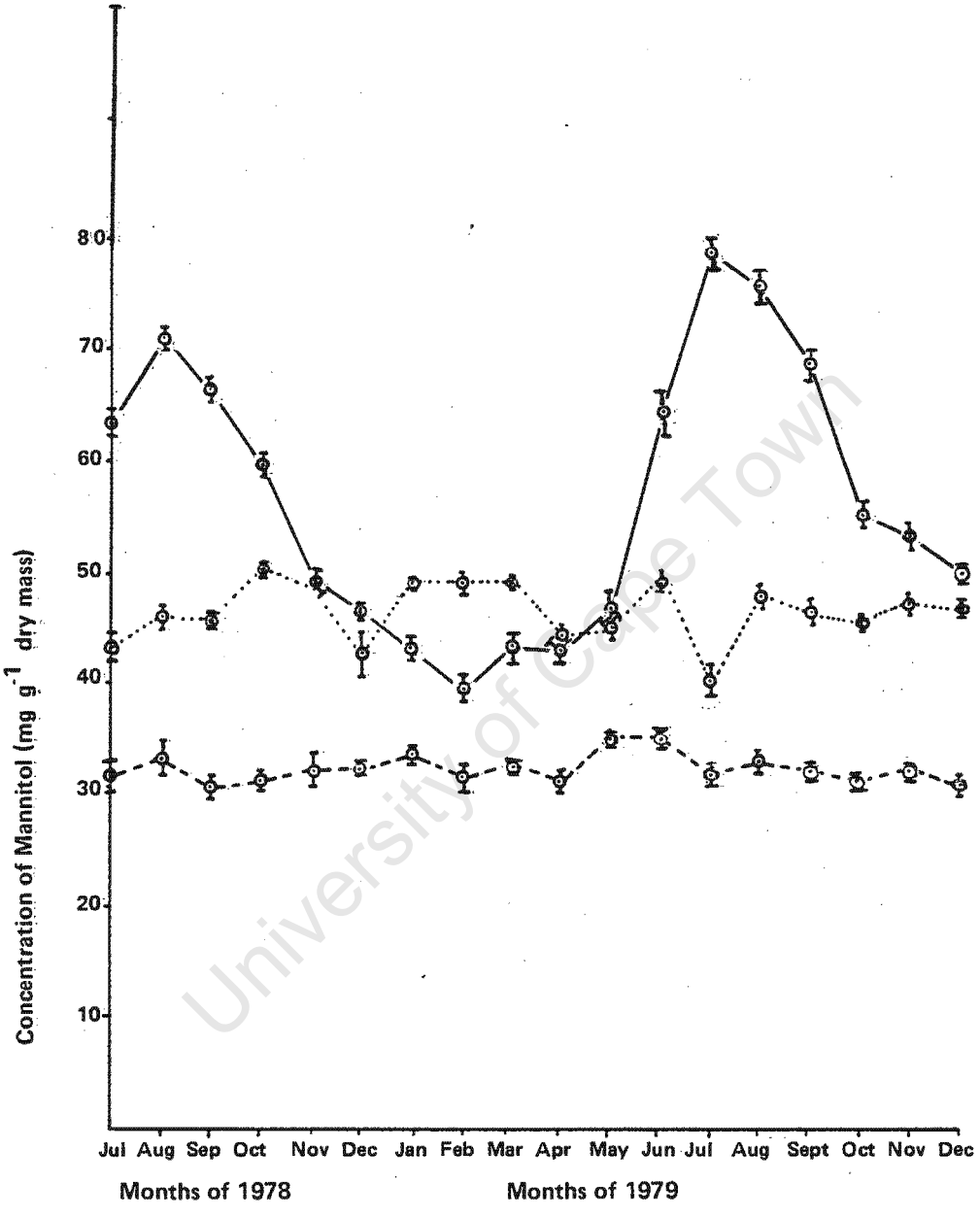


FIG.18 Seasonal variation of mannitol in the frond, meristematic region and stipe of *L. pallida*. Vertical bars represent twice the standard error of the mean; (·——·) frond; (·— — —) meristematic region; (····· ·) stipe.

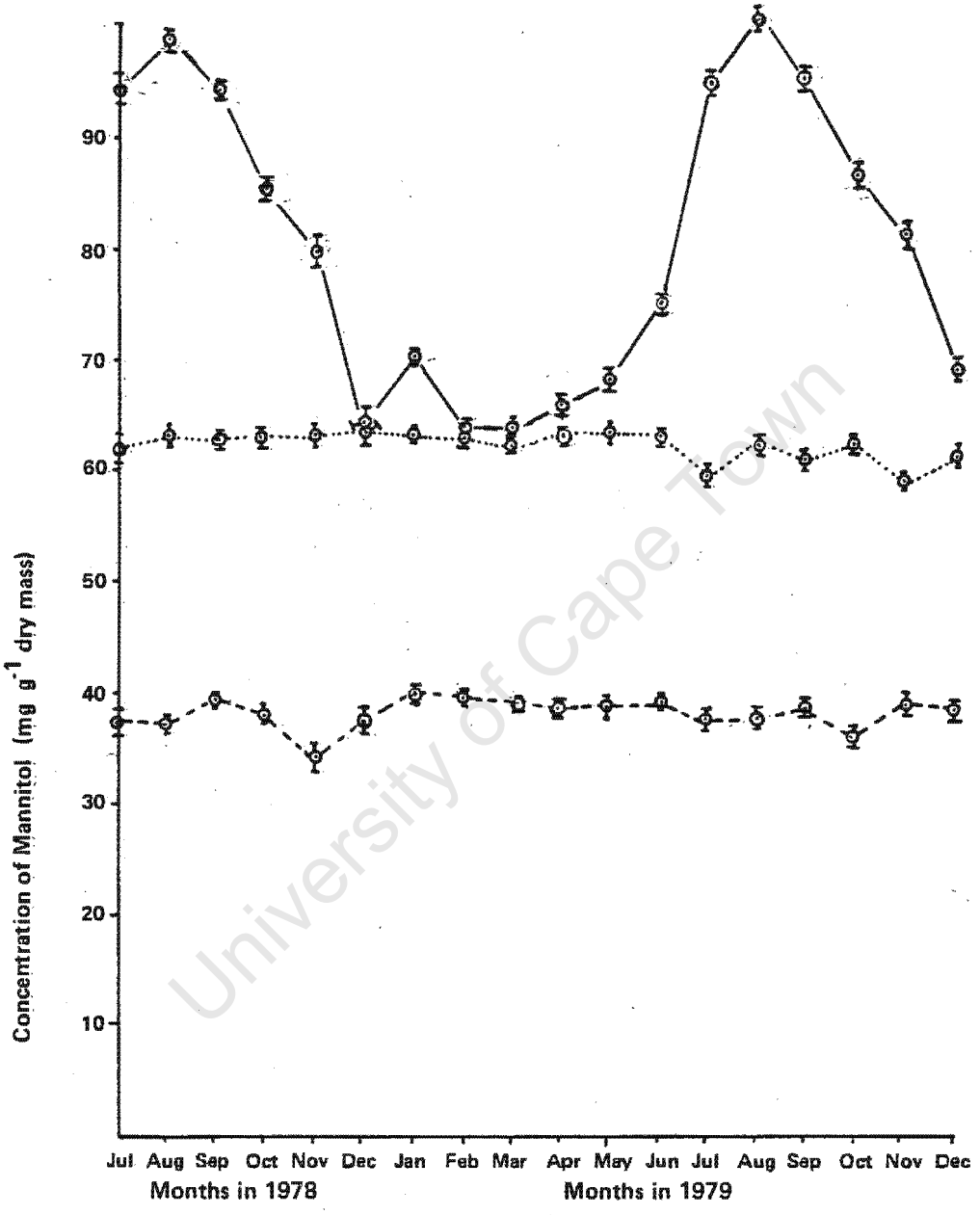


FIG.19 Seasonal variation of mannitol in the frond, meristematic region and stipe of *E. maxima*. Vertical bars represent twice the standard error of the mean; (•——•) frond; (– – –) meristematic region; (·····) stipe.

sample is the mean of four discs (17mm in diameter) taken at random from the frond and meristematic regions of four plants. A cork borer was used in the stipe to obtain 6 x 17mm discs of tissue from ten plants which were then trimmed to a depth of 3mm, the average frond thickness. The only marked seasonal variation in mannitol content occurred in the frond, whereas levels of this acyclic polyol were constant in the meristematic region and stipe throughout the investigation. Mannitol levels in the frond of *L. pallida* and *E. maxima* were consistently higher than in the stipe and meristematic region and reached a peak during the winter months. The mean concentration of mannitol over the eighteen months sampling period in *L. pallida* was 32,4 ± 1,23mg g^{-1} dry mass in the meristematic region, while in the stipe it was 46,2 ± 1,27mg g^{-1} dry mass. The mannitol content in the frond ranged from a low mean value of 43,0 ± 1,85mg g^{-1} dry mass in the summer months of December to February to a peak of 78,6 ± 1,67mg g^{-1} dry mass in July 1979. The mean concentration of mannitol over the sampling period in the meristematic region of *E. maxima* was 38,3 ± 1,27mg g^{-1} dry mass and in the stipe 63,6 ± 1,27mg g^{-1} dry mass. The amount of mannitol in the frond of *E. maxima* ranged from a mean value of 67,0 ± 1,21mg g^{-1} dry mass during the period December to February reaching a peak of 101,1 ± 1,47mg g^{-1} dry mass in August 1979. The mannitol content in all three regions sampled was consistently higher in *E. maxima* than in *L. pallida*.

3.6 Seasonal Variation in Laminaran

The laminaran content of the frond in both *L. pallida* and *E. maxima* changed seasonally, whereas no variations were found in either the stipe or meristematic region (Figs. 20 and 21 and Appendix Tables 5 and 6). The amount of laminaran in the stipe was negligible having a mean value over the eighteen months of sampling of 3,8mg g^{-1} dry mass in *L. pallida* and 5,3mg g^{-1} dry mass in *E. maxima*. The mean value over the sampling period in the meristematic region was 9,5mg g^{-1} dry mass in *L. pallida* and 13,2mg g^{-1} dry mass in *E. maxima*. The laminaran content in the frond ranged from a mean value

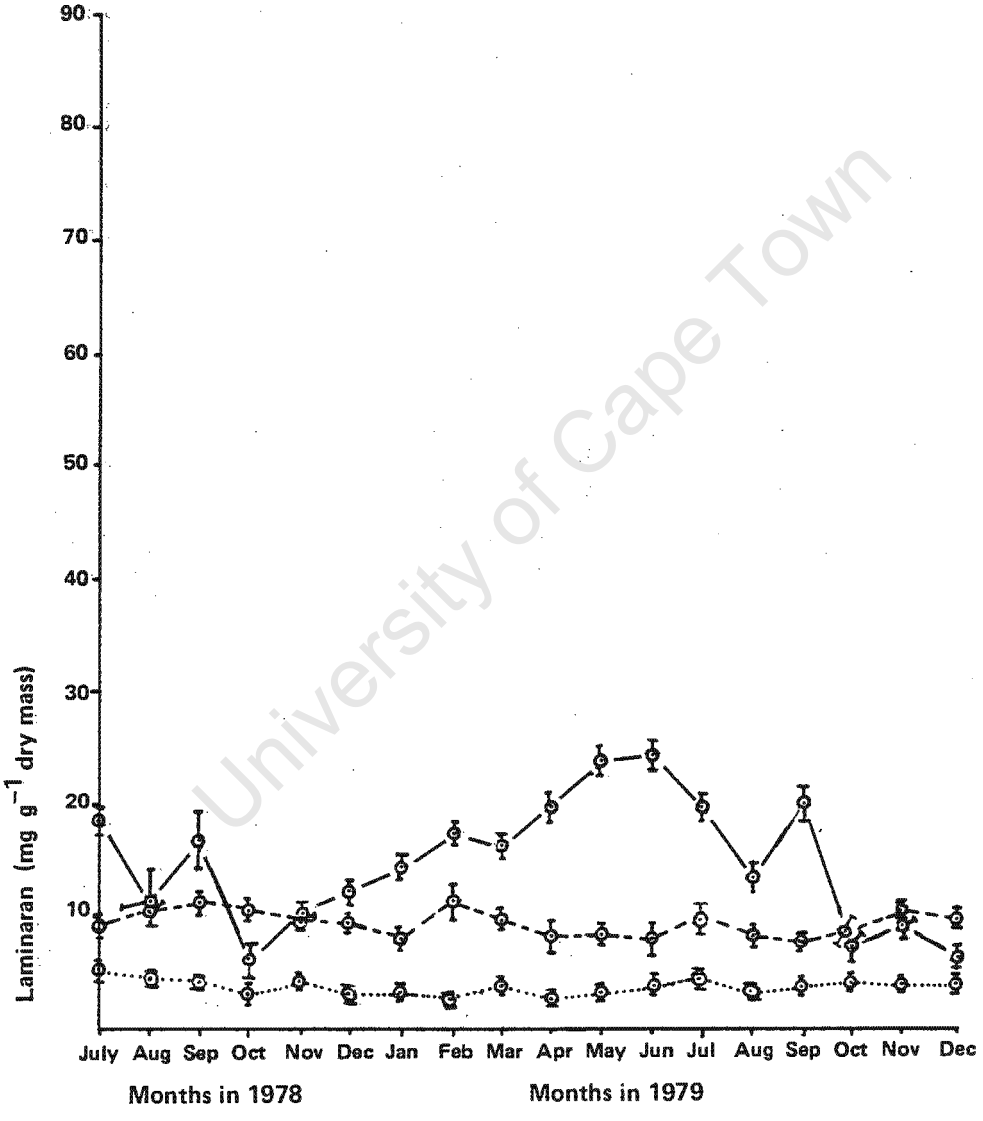


FIG. 20 Seasonal variation of laminaran in the frond, meristematic region and stipe of *L. pallida*. Vertical bars represent twice the standard error of the mean; (·——·) frond; (•——•) meristematic region; (·····) stipe.

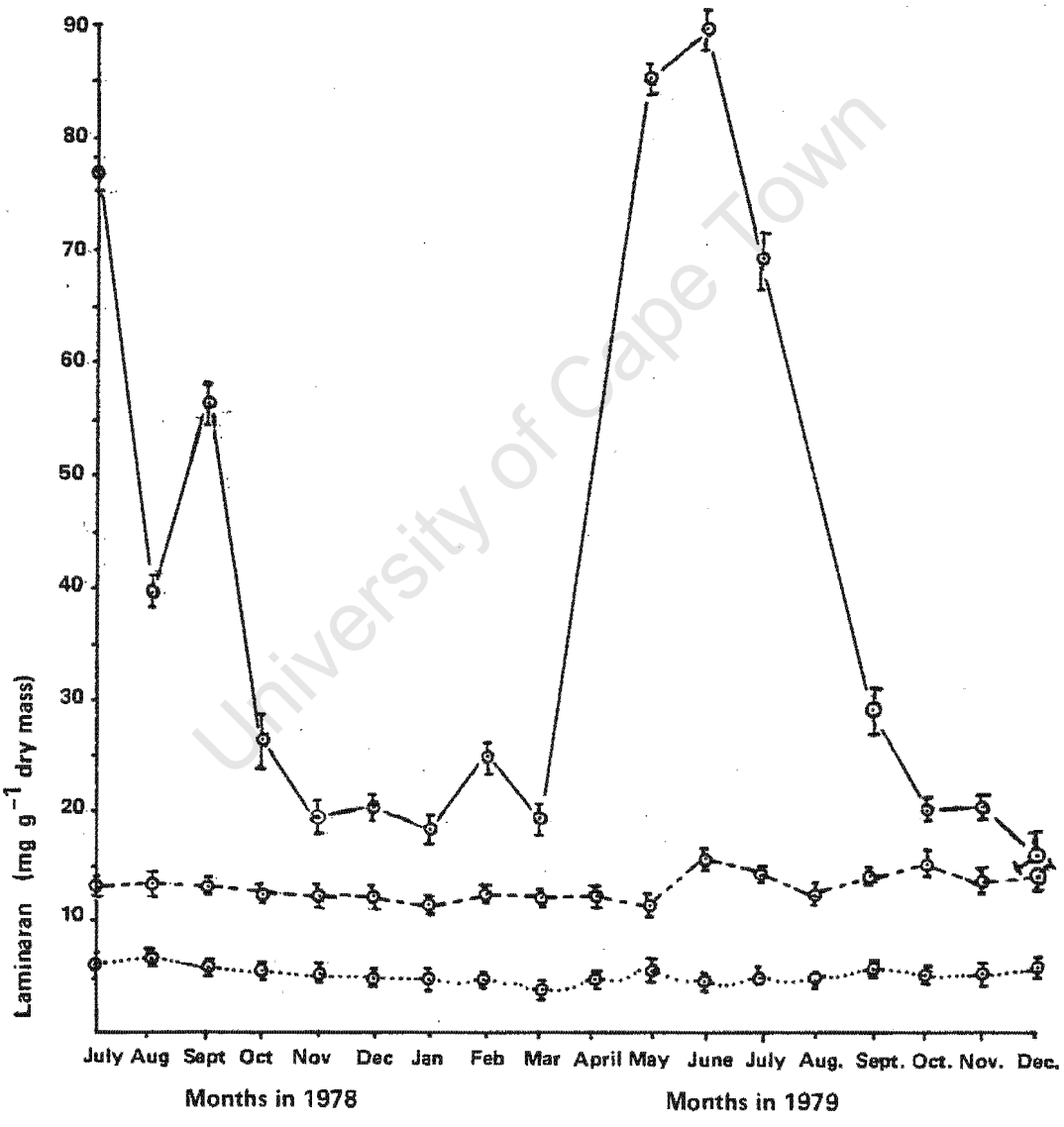


FIG.21 Seasonal variation of laminaran in the frond, meristematic region and stipe of *E. maxima*. Vertical bars represent twice the standard error of the mean; (·——·) frond; (•——•) meristematic region; (·····) stipe.

of 11,0 ± 0,35mg g^{-1} dry mass during the period November 1978 to January 1979 to 24,3 ± 1,48mg g^{-1} dry mass in June 1979 in *L. pallida*. The amount of laminaran in the frond of *E. maxima* varied from a mean value of 19,0 ± 0,16mg g^{-1} dry mass between November 1978 and January 1979 to 89,9 ± 1,52mg g^{-1} dry mass in June 1979. The amount of laminaran in all regions of *Ecklonia* was consistently higher than in *Laminaria*.

3.7 Seasonal Variation in Alginic Acid

No marked seasonal variation of alginic acid occurred in the meristematic region and stipe of either *L. pallida* or *E. maxima* but some seasonal changes were found in the fronds (Figs. 22 and 23 and Appendix Tables 7 and 8). The amount of alginic acid, on a dry mass basis, was higher in all three regions investigated in *E. maxima* than in the equivalent regions of *L. pallida*. There was no variation in the meristematic region of *L. pallida* with the average being 208mg g^{-1} dry mass during the eighteen months of the study. There was, however, a slight variation in the same region in *E. maxima* with a minor peak occurring during the summer. No seasonal variation was observed in the stipe of either kelp although there was an erratic pattern, especially in *E. maxima*, of the amount of alginic acid present in the stipe from month to month which tended to obscure any possible seasonal variation. The alginic acid content on a dry mass basis in both *L. pallida* and *E. maxima* increased in September to peak levels during the summer months but then decreased during the winter months of June and July. The highest value reached in *L. pallida* was 263,8 ± 1,29mg g^{-1} dry mass of alginic acid in December 1979 and the lowest value 195,7 ± 1,36mg g^{-1} dry mass in July 1978. The variations in the frond of *E. maxima* showed a range of a mean value of 279mg g^{-1} dry mass during the period September 1978 to March 1979 to a mean value of 231,5mg g^{-1} dry mass between the months of May 1979 and August 1979. There does not appear to be any significant difference between the concentration of alginic acid in the frond, stipe and meristematic

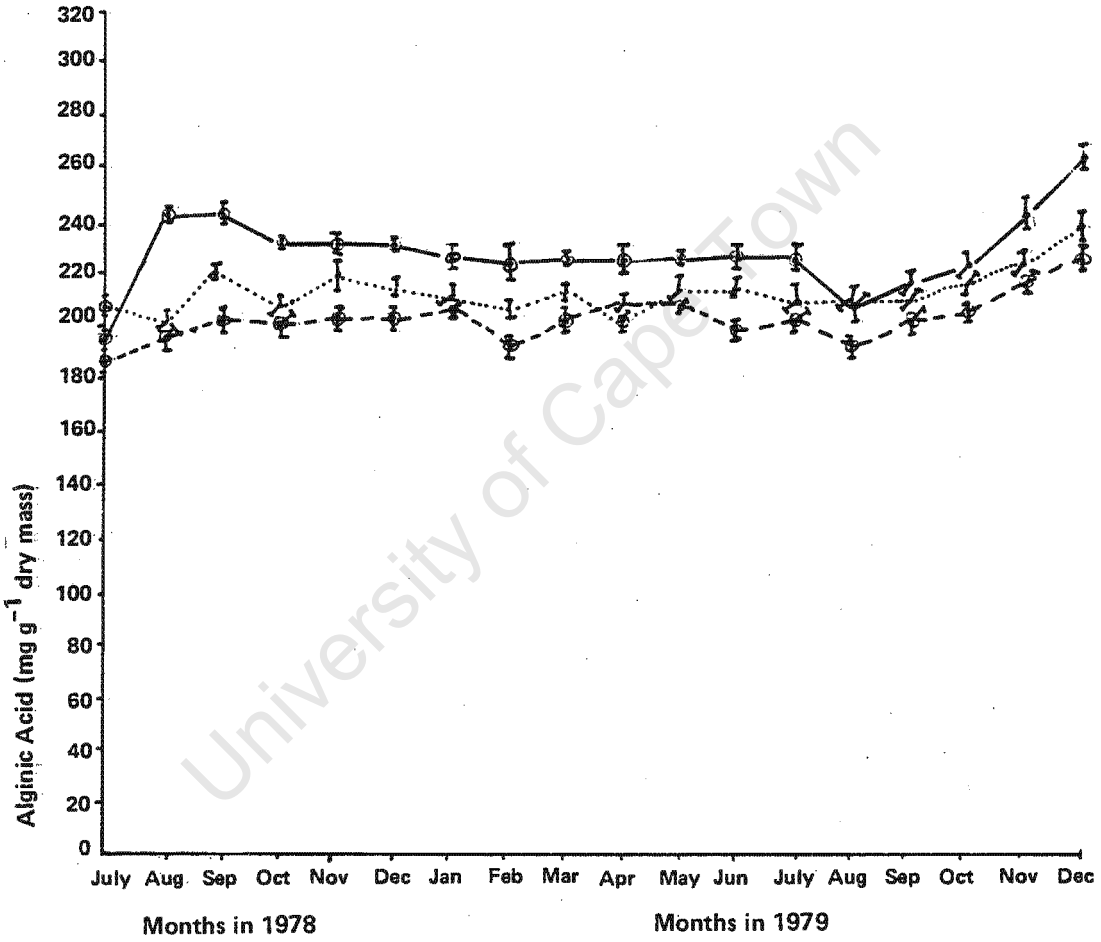


FIG. 22 Seasonal variation of alginic acid in the frond, meristematic region and stipe of *L. pallida*. Vertical bars represent twice the standard error of the mean; (· ——— ·) frond; (•------•) meristematic region; (· · · · · ·) stipe.

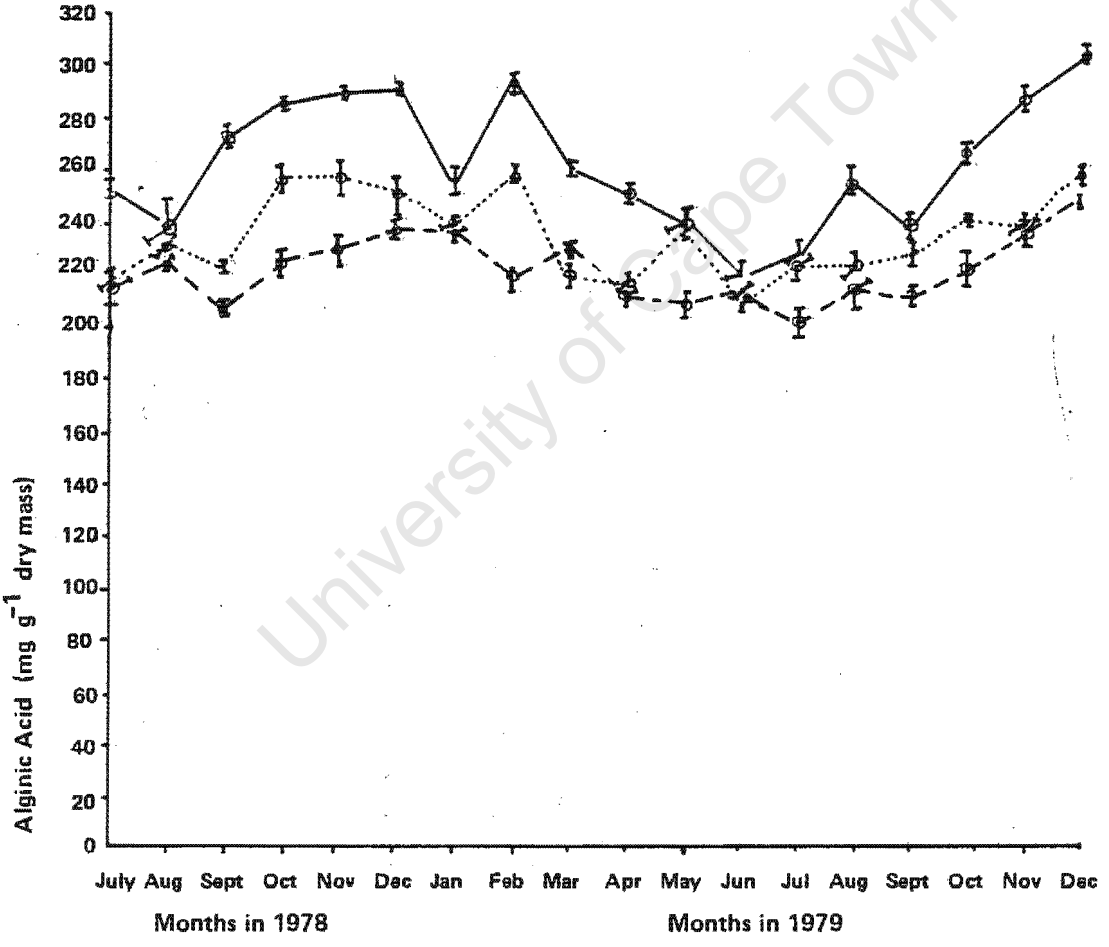


FIG. 23 Seasonal variation of alginic acid in the frond, meristematic region and stipe of *E. maxima*. Vertical bars represent twice the standard error of the mean; (·——·) frond; (— — —) meristematic region; (·····) stipe.

region.

3.8 Analysis of "Free" Amino Compounds and ^{15}N Incorporation

The "free" amino acids present in the kelp are shown in Table 4. The major "free" amino acids were alanine, glutamic acid and glutamine as well as aspartate and histidine. *Laminaria pallida* had a higher concentration of all these "free" amino acids than *E. maxima*.

The ^{15}N enrichment, concentration and ^{15}N content of the eight most common amino compounds and ammonia of *L. pallida* and *E. maxima*, after the incorporation of the isotope ^{15}N (described in section 2.3), are shown in Tables 5 and 6 respectively. The ^{15}N accumulation in both kelp was greater in glutamine than in all other amino compounds at all three stages of the time course. As assimilation proceeded all the amino compounds showed an increase in ^{15}N enrichment. The ^{15}N enrichment in the amino compounds was slightly higher in both brown algae when incubated with 400 $\mu g\ m\ell^{-1}$ concentration ^{15}N compared with the 200 $\mu g\ m\ell^{-1}$ concentration ^{15}N.

The results of feeding $K^{15}NO_3$ and the glutamine synthetase inhibitor MSO to both brown algae are shown in Tables 7 (*L. pallida*) and 8 (*E. maxima*). Comparison of the results in Tables 7 and 8 with those in Tables 5 and 6 showed that amino acid synthesis had almost stopped after the addition of MSO. There were large glutamine, alanine and glutamate pools in both kelp treated with MSO, but little, if any, enrichment in these amino compounds. The only compound which initially showed high enrichment was ammonia and, as the time course progressed, both the size of the ammonia pool and the amount of ^{15}N enrichment in the ammonia increased, while, in contrast, the pool size of the amino compounds decreased. (The abbreviations for the amino acids used in Tables 5 to 8 are given in Appendix Table 9).

TABLE 4

Concentration of "free" amino acids in µmoles g^{-1} fresh mass in *L. pallida* and *E. maxima*.

Amino Acid	*L. pallida*	*E. maxima*
Aspartic acid	0,498	0,313
Threonine	0,089	0,053
Serine	0,162	0,133
Asparagine	0,154	0,033
Glutamic acid	0,901	0,780
Glutamine	0,447	0,451
Glycine	0,129	0,240
Alanine	2,132	1,623
Valine	0,052	0,054
Cystine	0,093	0,003
Methionine	0,031	0,110
Isoleucine	0,017	0,017
Leucine	0,014	0,016
Tyrosine	0,017	0,039
Phenylalanine	0,074	0,053
Lysine	0,020	0,113
Histidine	0,761	0,376

TABLE 5

^{15}N enrichment and content of the commonest "free" amino compounds of *Laminaria pallida* after infiltration with $K^{15}NO_3$ for two, four and six hours

200 µg N mℓ$^{-1}$

	TWO HOURS			FOUR HOURS			SIX HOURS		
	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg$_1$ ^{15}N g^{-1} fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg$_1$ ^{15}N g^{-1} fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg$_1$ ^{15}N g^{-1} fm
ALA	0,610	1,230	0,113	0,690	2,571	0,266	0,640	1,309	0,126
ASN	0,260	0,147	0,115	0,300	0,235	0,021	0,420	0,105	0,013
ASP	0,280	0,047	0,002	0,300	0,128	0,006	0,430	0,290	0,019
GLN	1,540	0,992	0,458	2,130	1,382	0,883	2,400	2,489	1,792
GLU	1,330	1,095	0,218	1,640	1,133	0,279	1,640	1,783	0,439
GLY	0,340	0,123	0,004	0,620	0,158	0,015	0,630	0,360	0,034
THR	0,160	0,172	0,004	0,880	0,161	0,021	0,940	0,192	0,027
SER	0,260	0,087	0,003	0,860	0,038	0,005	0,670	0,019	0,002
NH_3	1,590	2,851	0,680	0,430	1,686	0,109	0,260	0,640	0,025

400 µg N mℓ$^{-1}$

	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg$_1$ ^{15}N g^{-1} fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg$_1$ ^{15}N g^{-1} fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg$_1$ ^{15}N g^{-1} fm
ALA	0,310	2,406	0,112	0,490	3,219	0,237	0,750	2,102	0,236
ASN	0,520	0,150	0,023	0,530	0,165	0,026	0,630	0,172	0,033
ASP	0,220	0,135	0,004	0,510	0,195	0,015	0,500	0,645	0,048
GLN	1,540	1,021	0,472	2,040	1,428	0,873	3,280	2,051	2,018
GLU	1,040	1,265	0,197	1,250	1,282	0,240	1,300	1,796	0,350
GLY	0,270	0,060	0,002	0,400	0,050	0,003	0,460	0,070	0,005
THR	0,510	0,165	0,013	0,520	0,298	0,023	0,570	0,825	0,071
SER	0,280	0,420	0,018	0,560	0,480	0,040	0,530	0,836	0,066
NH_3	1,240	4,540	0,844	0,950	1,930	0,275	0,120	1,480	0,027

g^{-1} fm = per gram fresh mass

TABLE 6

^{15}N enrichment and content of the commonest 'free' amino compounds of *Ecklonia maxima* after infiltration with $K^{15}NO_3$ for two, four and six hours

200 µg N $m\ell^{-1}$

	TWO HOURS			FOUR HOURS			SIX HOURS		
	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm
ALA	0,470	1,572	0,110	0,540	1,270	0,111	0,640	2,497	0,240
ASN	0,500	0,076	0,011	0,590	0,069	0,012	0,510	0,174	0,027
ASP	0,460	0,062	0,004	0,520	0,084	0,007	0,560	0,114	0,010
GLN	0,920	1,266	0,350	1,370	1,287	0,530	1,490	1,482	0,662
GLU	0,580	1,189	0,103	0,610	1,247	0,114	0,730	1,323	0,145
GLY	-	0,122	-	-	0,231	-	-	0,283	-
THR	0,590	0,066	0,006	0,580	0,134	0,012	0,590	0,132	0,012
SER	0,520	0,244	0,019	0,530	0,226	0,018	0,530	0,360	0,029
NH_3	3,640	2,805	1,532	2,870	1,590	0,684	0,510	0,567	0,043

400 µg N $m\ell^{-1}$

	TWO HOURS			FOUR HOURS			SIX HOURS		
	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm
ALA	0,530	2,400	0,191	0,520	2,718	0,212	0,790	3,202	0,382
ASN	0,140	0,083	0,004	0,320	0,124	0,012	0,510	0,251	0,038
ASP	0,260	0,142	0,006	0,410	0,346	0,021	0,470	0,662	0,047
GLN	0,880	1,445	0,381	1,960	1,473	0,866	2,120	1,593	1,013
GLU	0,590	1,362	0,120	1,350	1,556	0,315	2,050	1,748	0,538
GLY	-	0,023	-	-	0,065	-	0,130	0,070	-
THR	0,470	0,105	0,007	0,500	0,122	0,009	0,650	0,142	0,014
SER	0,410	0,109	0,007	0,540	0,369	0,030	0,630	0,373	0,035
NH_3	2,630	5,045	1,990	1,170	3,950	0,871	0,670	1,687	0,170

g^{-1}fm = per gram fresh mass

TABLE 7

^{15}N enrichment and content in the commonest 'free' amino compounds of *L. pallida* after infiltration with $K^{15}NO_3$ and 5 mM MSO for four and six hours.

200 µg N $m\ell^{-1}$

	FOUR HOURS			SIX HOURS		
	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	^{15}N content μg_1 15N g fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	^{15}N content μg_1 15N g fm
ALA	0,032	1,283	0,006	0,000	0,716	0,000
ASN	0,014	0,135	0,000	0,000	0,093	0,000
ASP	0,057	0,455	0,004	0,000	0,210	0,000
GLN	0,113	0,942	0,032	0,080	0,611	0,015
GLU	0,073	0,633	0,007	0,000	0,386	0,000
GLY	0,000	0,166	0,000	0,000	0,073	0,000
SER	0,000	0,285	0,000	0,000	0,125	0,000
THR	0,010	0,268	0,000	0,000	0,091	0,000
NH_3	1,334	1,743	0,349	2,370	5,070	1,802

400 µg N $m\ell^{-1}$

	FOUR HOURS			SIX HOURS		
	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content μg_1 15N g fm
ALA	0,020	2,102	0,006	0,000	0,000	0,000
ASN	0,010	0,135	0,000	0,000	0,007	0,000
ASP	0,050	0,296	0,002	0,000	0,142	0,000
GLN	0,090	0,651	0,018	0,100	0,321	0,010
GLU	0,070	0,437	0,005	0,000	0,137	0,000
GLY	0,000	0,114	0,000	0,000	0,013	0,000
SER	0,000	0,167	0,000	0,000	0,045	0,000
THR	0,020	0,149	0,000	0,000	0,041	0,000
NH_3	1,200	4,917	0,885	1,935	10,324	2,997

g^{-1} fm = per gram fresh mass

TABLE 8

^{15}N enrichment and content in the commonest 'free' amino compounds of *Ecklonia maxima* after infiltration with $K^{15}NO_3$ and 5 mM MSO for four and six hours

200 µg N $m\ell^{-1}$

	FOUR HOURS			SIX HOURS		
	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	^{15}N content µg ^{15}N g^{-1} fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	^{15}N content µg ^{15}N g^{-1} fm
ALA	0,010	2,219	0,003	0,000	1,203	0,000
ASN	0,010	0,830	0,002	0,000	0,013	0,000
ASP	0,010	0,387	0,000	0,000	0,219	0,000
GLN	0,090	0,521	0,014	0,030	0,328	0,003
GLU	0,080	0,365	0,004	0,000	0,282	0,000
GLY	0,000	0,012	0,000	0,000	0,010	0,000
SER	0,000	0,184	0,000	0,000	0,096	0,000
THR	0,000	0,233	0,000	0,000	0,198	0,000
NH_3	0,970	2,597	0,378	1,180	5,016	0,888

400 µg N $m\ell^{-1}$

	FOUR HOURS			SIX HOURS		
	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg 15N g^{-1} fm	Enrichment (A%E)	Concentration in frond µ moles g^{-1}fm	15N content µg 15N g^{-1} fm
ALA	0,010	1,017	0,001	0,000	0,793	0,000
ASN	0,010	0,092	0,000	0,000	0,074	0,000
ASP	0,020	0,703	0,002	0,000	0,185	0,000
GLN	0,120	0,824	0,030	0,020	0,503	0,003
GLU	0,080	0,626	0,008	0,000	0,371	0,000
GLY	0,000	0,458	0,000	0,000	0,266	0,000
SER	0,010	0,327	0,000	0,000	0,198	0,000
THR	0,000	0,134	0,000	0,000	0,126	0,000
NH_3	1,060	5,322	0,848	1,250	9,936	1,863

g^{-1} fm = per gram fresh mass

3.9 Discussion

3.9.1 Translocation and Anatomy

In the ^{14}C-assimilation studies the unique design of the perspex chambers in which the plants were incubated for six hours (Fig. 8 section 2.2) allowed the frond to be exposed to seawater containing ^{14}C-sodium bicarbonate, while the stipe was immersed in non-radioactive seawater. Various control experiments were carried out to show that movement was within the kelp and not via some other means. Analysis of the seawater around the stipe gave only slightly higher counts than untreated seawater. This demonstrated that the ^{14}C-label was not transferred due to leakage of seawater from the upper to the lower chamber. Seawater from around the stipe of kelp yielded counts of less than 2 000 DPM for the bottom chamber (Fig. 8) with a volume of 20ℓ. Untreated seawater was found to yield counts of approximately half this value which was due to salts in the seawater interfering with the counting efficiency. These results indicated that secretion of the ^{14}C-label by the kelp into the seawater was negligible in this study. The values reported for dissolved organic carbon losses by macrophytes are very variable. The *in vitro* experiments of Sieburth (1969) suggested that up to 45% of photosynthetically fixed carbon was released by *Fucus vesiculosus* L. and up to 40% by two species of *Laminaria* into their surrounding seawater. Khailov and Burlakova (1969) found that 37% of fixed carbon was exuded from various marine algae including *Ascophyllum nodosum*. Moebus and Johnson (1974) and Moebus *et al.* (1974) showed that in *A. nodosum* and *F. vesiculosus* excretion of dissolved organic substances was undetectable except after a period of dessication. Brylinski (1977) reported a value of 1-4% of total carbon fixed was released into the surrounding water in several species of macroalgae, whilst Harlin and Craigie (1975) found only 0,3-1,5% of photoassimilated carbon released as dissolved organic matter. Hatcher *et al.* (1977) have estimated that as much as 35% of the assimilated carbon in a kelp is released as dissolved organic material and a carbon budget for *L. saccharina* calculated that for an annual input of carbon of 100g approximately 13g carbon per year

was lost as extracellular products (Johnson *et al.*, 1977) Under the conditions used in this investigation the amount of photoassimilated carbon lost by *L. pallida* and *E. maxima* was very small. All plants in this study which exuded large quantities of mucilage were discarded as these plants would have been difficult to seal in the neoprene rubber separating the two chambers. In the experiments using the perspex chamber and attached ampoule (section 3.2) translocation of ^{14}C-labelled assimilates was shown to occur in *L. pallida* and *E. maxima*. The velocity of movement was also determined; *E. maxima* translocated ^{14}C-assimilates more rapidly than *L. pallida*. The velocity of translocation in *L. pallida* and *E. maxima* agreed with the results obtained for other members of the Laminariales (Table 9 is a summary). Slowest velocities of translocation were demonstrated in the Laminareaceae and Alariaceae. Fastest velocities were observed in the Lessoniaceae which includes the genera *Macrocystis* and *Nereocystis* (Table 9). This may be due to the presence of sieve elements and sieve pores in the medulla of the sporophytes in the Lessoniaceae which are wider than those in the Laminareaceae and Alariaceae (Parker and Huber, 1965; Schmitz and Srivastava, 1976; Ziegler, 1963). The results summarized in Table 9 were calculated in the same manner and were derived from "pulse chase" labelling experiments. Canny (1960) in a review on higher plants, stated that velocity calculated in this manner was dependent on the initial dose of radioactivity, the apparent velocity at higher doses being greater than at lower ones. Buggeln (1976) measured the rate of translocation of organic carbon in *Alaria esculenta* (L.) Grev. and values computed for specific mass transfer of carbon into the blade meristem ranged from 36,2 to 60,8mgC $wk^{-1}0,1mm^{-2}$ sieve filaments, but problems have been encountered using this method (Buggeln, 1976).

These initial ^{14}C-labelling experiments on *L. pallida* and *E. maxima* were necessary to demonstrate movement from frond to stipe and to establish the correct time span for the experiments to allow this movement to occur. The majority of the ^{14}C-activity which was exported accumulated in the stipe (Table 1) and could only have been transported from

TABLE 9

Velocity of translocation of ^{14}C-labelled assimilates in certain brown algae.

Algal Family	Algal Genus and species	Translocation velocity in mm h^{-1}	Authors
Laminariaceae	*L. pallida*	50 - 100	Stacey (this study)
	L. setchellii	55	Schmitz and Lobban (1976)
	L. hyperborea	55	Lüning *et al.* (1972)
	L. saccharina	55	Lüning *et al.* (1972)
	L. digitata	170	Buggeln (1981)
	L. digitata	100	Hellebust and Haug (1972)
	S. dermatodea	60 - 100	Emerson *et al.* (1982)
Lessoniaceae	*M. pyrifera*	650 - 780	Parker (1965, 1966)
	M. integrifolia	350 - 720	Schmitz and Srivastava (1979a)
	N. lutkeana	370	Nicholson and Briggs (1972)
	N. lutkeana	570	Schmitz and Lobban (1976)
Alariaceae	*E. maxima*	240 - 300	Stacey (this study)
	A. marginata	250 - 400	Schmitz and Lobban (1976)

the frond. Lüning *et al.* (1972) found that the activity accumulated in the base of the frond of *L. hyperborea* and *L. saccharina* after an incubation of ninety six hours. The proportion of total ^{14}C-label in the base of the frond was 71% in *L. hyperborea* and 63% in *L. saccharina*. Schmitz and Lobban (1976) concluded that long distance transport of assimilates was a general phenomenon in the Laminariales from mature frond areas to "sinks" which were either growing regions or regions deficient in assimilates. Schmitz and Srivastava (1979a) suggested that *Macrocystis* was the only member of the Laminariales which exuded cell sap in any quantity. In this study attempts to obtain exudate from *E. maxima* and *L. pallida* were unsuccessful. Lobban (1978) also found a source/sink relationship in *M. integrifolia* whereby mature blades (closer to the holdfast) together with secondary and tertiary fronds transported to the base, blades nearest the apex transported to the apex and the intermediate blades transported both acropetally and basipetally. *Laminaria pallida* and *E. maxima* exhibited a velocity of translocation comparable to other members of the Laminariales (Table 9). Stephenson *et al.* (1976) demonstrated that there was a positive correlation between photosynthetic activities and translocation rates in higher plants. The movement appeared to be faster in *E. maxima* (Fig. 15) and slower in *L. pallida* (Fig. 14). Schmitz and Lobban (1976) state a velocity more than 50mm h^{-1} is greater than diffusion and *L. pallida* gave rates of 50-100mm h^{-1}. *Ecklonia maxima* possessed branched sieve elements with a larger diameter than *L. pallida* and it would be expected that this system could translocate faster than the unbranched sieve elements of *L. pallida*. Only in *L. pallida* was the distinctive trumpet shape of a sieve element observed (Pl.2A).

Sieve element equivalents have been observed in the majority of the Laminariales (Sideman and Scheirer , 1977). Sykes (1908) found "sieve tubes" in the inner cortex of *L. saccharina* whereas Oliver (1887) and Parker and Huber (1965) stated that only the Lessoniaceae possessed true "sieve tubes". The sieve elements of the Laminariaceae were not as large as, nor possessed, the well differentiated sieve plates of

the Lessoniaceae. Fritsch (1945) claimed that the trumpet hyphae of the Laminariales and sieve tubes of the Lessoniaceae were conducting elements, while Rigg (1925) claimed that they were specifically concerned with transport of protein. The use of the electron microscope has yielded more data, but fixation procedures in $KMnO_4$ have produced poor results (Ziegler and Ruck, 1967). Went and Tammes (1973) stated that the trumpet shape could be an artifact caused by the loss of turgor when the tissue was sectioned for microscopy, but other workers (Schmitz and Srivastava, 1974) disagreed with this interpretation, the trumpet shape being due to elongation of the sieve elements while the diameter at the cross walls remained constant (Schmitz and Srivastava. 1974). The ultrastructure of *L. groenlandica* Rosenv. (Schmitz and Srivastava, 1974), *Alaria marginata* (Schmitz and Srivastava, 1975) and *Nereocystis luetkeana* (Schmitz and Srivastava, 1976) has provided evidence that the conducting cells of certain Laminariaceae, Alariaceae and Lessoniaceae may be classified on a scale ranging from primitive to advanced algal sieve elements (Schmitz and Srivastava, 1976). The sieve areas of *Macrocystis* resemble those in higher plants with cell walls which are perforated by plasmodesmata like tubes (Parker and Philpott, 1961). A great deal of morphological variation, due to environmental conditions, has been recognised in kelp plants and the criteria used in classification of *Laminaria* has been reviewed by Dawson (1966). Mucilage ducts are not present in all species of *Laminaria* but Lüning *et al.* (1972) have demonstrated translocation does occur in the Laminariaceae as it does in the Lessoniaceae even though *Laminaria* plants are smaller and lack enucleate sieve tubes and microscopically visible pores (Schmitz and Srivastava, 1974). Lüning *et al.* (1972) demonstrated that translocation was from the distal part of the blade to the basal part and holdfast.

Analysis of the ethanol-soluble fraction of the kelp using paper chromatography and autoradiography, localised the ^{14}C-label as being the acyclic polyol mannitol after six hours of photosynthesis and translocation. Mannitol is known to be the major product of photosynthesis in the kelp

(Craigie, 1974). The other compounds which carried the ^{14}C-label were certain amino acids, the most heavily labelled being alanine, aspartate and asparagine.

The identification of mannitol as the major product of translocation agreed with the findings of other workers. Parker (1966) and Schmitz and Srivastava (1979a, 1979b) also found ^{14}C-labelled mannitol and amino acids in the whole plant of *M. integrifolia*, and similar amino acids were identified e.g., aspartate, glutamate, glycine, serine and alanine. Schmitz and Srivastava (1979a) obtained 10% w/w mannitol which was slightly higher than the concentration found in this investigation. Parker (1966) however found a lower mannitol concentration (36mg $m\ell^{-1}$) but used totally different methods of analysis to Schmitz and Srivastava (1979a). Schmitz *et al.* (1972) demonstrated that the components of the translocated ^{14}C-label in *L. hyperborea* and *L. saccharina* consisted of mannitol (53%), the amino acids, alanine, glycine, serine, glutamic acid and others (45%), and malate (2%). This composition was similar to the products of photosynthesis in *L. hyperborea* and *L. saccharina*. The results in this chapter indicate that the radioactive amino acids in *L. pallida* and *E. maxima* were glycine, serine, glutamic acid, alanine, aspartate and asparagine. Schmitz and Lobban (1976) in a review on translocation in the Laminariales, found the translocate was mainly mannitol and some amino acids which were rapidly metabolised and incorporated at the growing region into polysaccharides and proteins.

The autoradiographs of the chromatograms of the ethanol-soluble carbohydrates showed the presence of only one radioactive component which was identified as mannitol. It was impossible to prove whether the amino acids were translocated or synthesised *in situ* from a ^{14}C-labelled precursor.

3.9.2 Seasonal Variation of Mannitol, Laminaran and Alginic Acid

The results presented in this investigation on the seasonal variation in mannitol (section 3.5), laminaran (section 3.6) and alginic acid (section 3.7) agree with the findings of

von Holdt *et al.* (1955). The frond was the only region showing any variation with mannitol and laminaran at a maximum concentration in winter and a minimum in summer. Alginic acid was at a maximum concentration in summer and a minimum in winter.

In the South-western Cape waters *L. pallida* and *E. maxima* grow actively during July/early August at a time of the year when high levels of mannitol are photosynthesised. Laminaran may be formed from mannitol (Percival and McDowell, 1967) so that during active growth large quantities of mannitol are formed and converted to laminaran. During active growth mannitol synthesis is more rapid than is its conversion to laminaran, but as growth ceases this may not be true resulting in a slight increase in the amount of laminaran relative to the mannitol content. Mann *et al.* (1979) showed that in July/August *E. maxima* plants were growing at the rate of 1% per day. This active winter growth would indicate rapid mannitol synthesis and conversion to laminaran explaining its maximum in winter. The mannitol and laminaran concentration would drop and the alginic acid would reach its summer maximum as growth slows down.

Increase in the proportion of dry solids made up of mannitol, salts of alginic acid and laminaran occur during further photosynthesis in tissue undergoing cell enlargement (Percival and McDowell, 1967). Cellulose and protein are also being synthesised. In each unit of tissue, mannitol and laminaran formation continues after the other constituents have built up to a constant level thus increasing the dry solids content and reducing the proportion of alginate, cellulose and protein on a dry mass basis. This would explain the high levels of mannitol and laminaran in the kelp studied in May and June before active growth has started. The peak of laminaran occurred in June and that of mannitol in July. Mannitol and laminaran would also be utilized in periods of spore formation or when respiration exceeds photosynthesis (Percival and McDowell, 1967). During the summer months the kelp appear to go into a quiescent state but no data exists on the rates of photosynthesis versus respiration

in these kelp. Limited data on the time of sporogenesis in these kelp exist but from observed results it appears that *E. maxima* is fertile all the year round and *L. pallida* towards the end of summer, i.e., from January to April (Dieckmann, 1978). During sporogenesis and periods when respiration is greater than photosynthesis, both laminaran and mannitol are used up (Percival and McDowell, 1967). Laminaran constitutes an important component of the dry mass of many brown algae. Powell and Meeuse (1964) found values ranging from less than 2% up to 34% of the dry mass was laminaran in nineteen northern hemisphere species (sixteen genera). To eliminate possible errors due to variation in dead material, results may be expressed on a living material basis.

3.9.3 ^{15}N-Incorporation

The results in Tables 5 and 6 (Chapter 3, section 3.8) indicate that the compound which showed the greatest increase in ^{15}N-enrichment was glutamine for both plants at both ^{15}N-concentrations and at all three incubation times. The amino compounds with high pool size in both kelp were alanine, glutamine and glutamate. Despite its high pool size glutamine showed the highest ^{15}N-enrichment. Tables 5 and 6 show that the amount of ^{15}N-enrichment in the amino compounds was greatest in those samples with the longest incubation time (six hours) and least in those with the shortest incubation time (two hours) while ^{15}N-enrichment was greatest at the higher ^{15}N-concentration to which the plants were exposed.

The evidence from the inhibitor methionine sulphoximine (MSO) and ^{15}N-potassium nitrate incorporation studies reported in Tables 7 and 8 (Chapter 3, section 3.8) shows that both these kelp probably assimilate nitrate via the GS/GOGAT pathway. This became clear when it was found that the blocking of the pathway by MSO resulted in a build up of unassimilated ammonia and a decrease in the pool size of amino acids (as these were being utilized in metabolism and were not being replaced) and a complete cessation of ^{15}N entering into amino acid assimilation. It would appear that GDH plays no part in nitrate assimilation in the brown algae *L. pallida* and *E. maxima*. There were probably two

pools of ammonium ions with a small metabolic pool in the plants which quickly became saturated and a larger pool derived from protein breakdown. For this reason it was difficult to draw any conclusions from the ammonium ion pool size. The pathway of nitrate assimilation in the kelp *L. pallida* and *E. maxima* would appear to be via the GS/GOGAT enzyme system. This agrees with the results of Haxen and Lewis (1981) working on *Macrocystis angustifolia* who found the major route of $^{15}NO_3$ was to glutamine followed by a secondary enrichment to glutamate: incorporation ceased on the addition of MSO. Methionine sulfoximine is an analogy of the γ-glutamyl-phosphate enzyme complex of glutamine synthetase (Tate and Meister, 1973) and is an irreversible inhibitor of glutamine synthetase but has no effect on glutamine dehydrogenase activity (Miflin and Lea, 1976).

CHAPTER 4

PHYSIOLOGICAL INVESTIGATION OF *CARPOBLEPHARIS MINIMA* AND *SUHRIA VITTATA*

4.1 Introduction

Prior to this study, there was no information on the physiology of *Carpoblepharis minima* and *Suhria vittata*. In order to make an analysis of the kelp/red algal relationship, it was necessary to obtain data on those aspects of physiology of the red algae relevant to this association. Mannitol is well documented as a major component, being an important photosynthetic product (Craigie, 1974) and translocant in brown algae including *L. pallida* and *E. maxima* (see Chapter 3). Mannitol is not a common component of red algae and Kremer (1976a) states that many identifications of mannitol in red algae were incorrect. Mannitol has only been found to accumulate in the Ceramiales (Craigie, 1974) the class to which *C. minima* belongs, where the algal storage product floridoside is not present in any quantity (Craigie, 1974). Other storage products identified in the Ceramiales include fructose and trehalose in *Batrachospermum* Roth (Majak *et al.*, 1966); sucrose and trehalose in *Callithamnion tetricum* Lyngbye (Craigie, 1974); fructose in *Ceramium rubrum* (Huds.) J. Ag. (Majak *et al.*, 1966) and sodium mannoglycerate and trehalose in *Delesseria sanguinea* (Craigie, 1974). Majak *et al.* (1966) have identified the presence of mannitol in nine species of red algae, but other polyols do not appear to occur in the red algae (Craigie, 1974). In this study, *C. minima* and *S. vittata* were exposed to ^{14}C-labelled mannitol to determine its incorporation. Although mannitol may be passively moved across from the brown algal partner, this does not establish its utilization by the red algae. It was beyond the scope of this investigation to study in detail the fate of the mannitol after incorporation. *Carpoblepharis minima* and *S. vittata* appear to possess all the photosynthetic pigments having the typical colour of red algae, but prior to this investigation, the pigments

of these two red algae had not been identified. The pigmentation of algal parasites varies, *Holmsella* is white with reduced plastids, whereas *Janczweskia* is white or pink (depending on the species) with plastids present (Nonomura, 1979). The Rhodophyceae have the accessory photosynthetic pigments the phycobilins (phycocyanin and phycoerythrin) as well as chlorophylls.

4.2 Assimilation of ^{14}C-labelled Assimilates

The results of a time course study of the incorporation of ^{14}C-label by the red algae attached to a small portion of brown algal stipe, are shown in Fig. 24 for *C. minima* and Fig. 25 for *S. vittata* and indicated that these red algae were able to incorporate the ^{14}C-label. (The data from which these figures were plotted is given in Appendix Tables 10 and 11 respectively). The combined samples referred to in Figs. 24 and 25 were sections taken from both brown algae and their attached red algae on a dry mass basis in a ratio of 3:1 (brown:red). The ^{14}C-activity was greater in *Suhria* than in *Carpoblepharis* and in both *C. minima* and *S. vittata* the ^{14}C-activity was higher in the unattached red algae (A in Fig. 24 and A in Fig. 25 respectively) than in the red algae attached to its brown algal partner (B in Fig. 24 and B in Fig. 25 respectively). Portions of stipe of *L. pallida* and *E. maxima* (C in Fig. 24 and C in Fig. 25 respectively) contained the least ^{14}C-activity after sixteen hours incubation in ^{14}C-label. This supports the premise that the stipe is not a major photosynthetic region in the brown algae.

The method of Callow *et al.* (1979) yielded the distribution of ^{14}C-label in the ethanol-soluble, acid-hydrolysate and starch-containing fractions after exposure of both red algae to exogenously supplied mannitol (Table 10). Analysis of the total activity due to ^{14}C-mannitol in the seawater surrounding the red algae, on a scintillation counter, prior to the start, and at the end, of the experiment, showed that *S. vittata* had removed 4% of the total activity, but *C. minima* had removed 17% over a twenty four hour period. The percentage of ^{14}C-label in each fraction of *C. minima* remained relatively constant over the twenty four hour period of the experiment with over 80% of the ^{14}C-label in the ethanol-soluble fraction (Table 10). In the case of *S. vittata*,

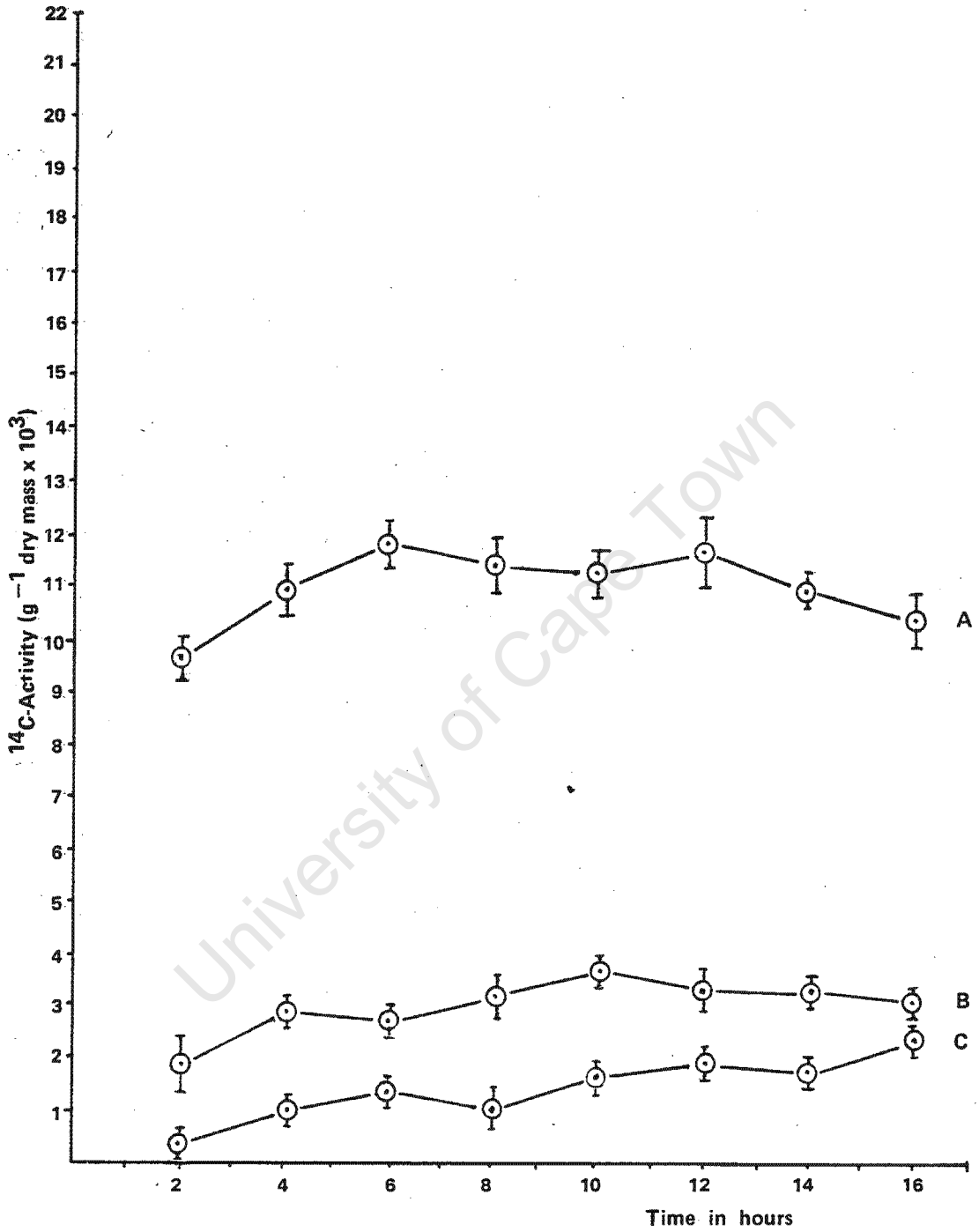


FIG. 24 A time course of the ^{14}C-labelled assimilates after incubation in NaH$^{14}CO_3$ seawater in *C. minima*.

A = *C. minima*

B = combined samples of whole plants of *C. minima* and the portions of stipe of *L. pallida* to which they were attached.

C = stipe of *L. pallida* with no attached red algae

Results are means of six replicates and vertical bars represent twice the SEM.

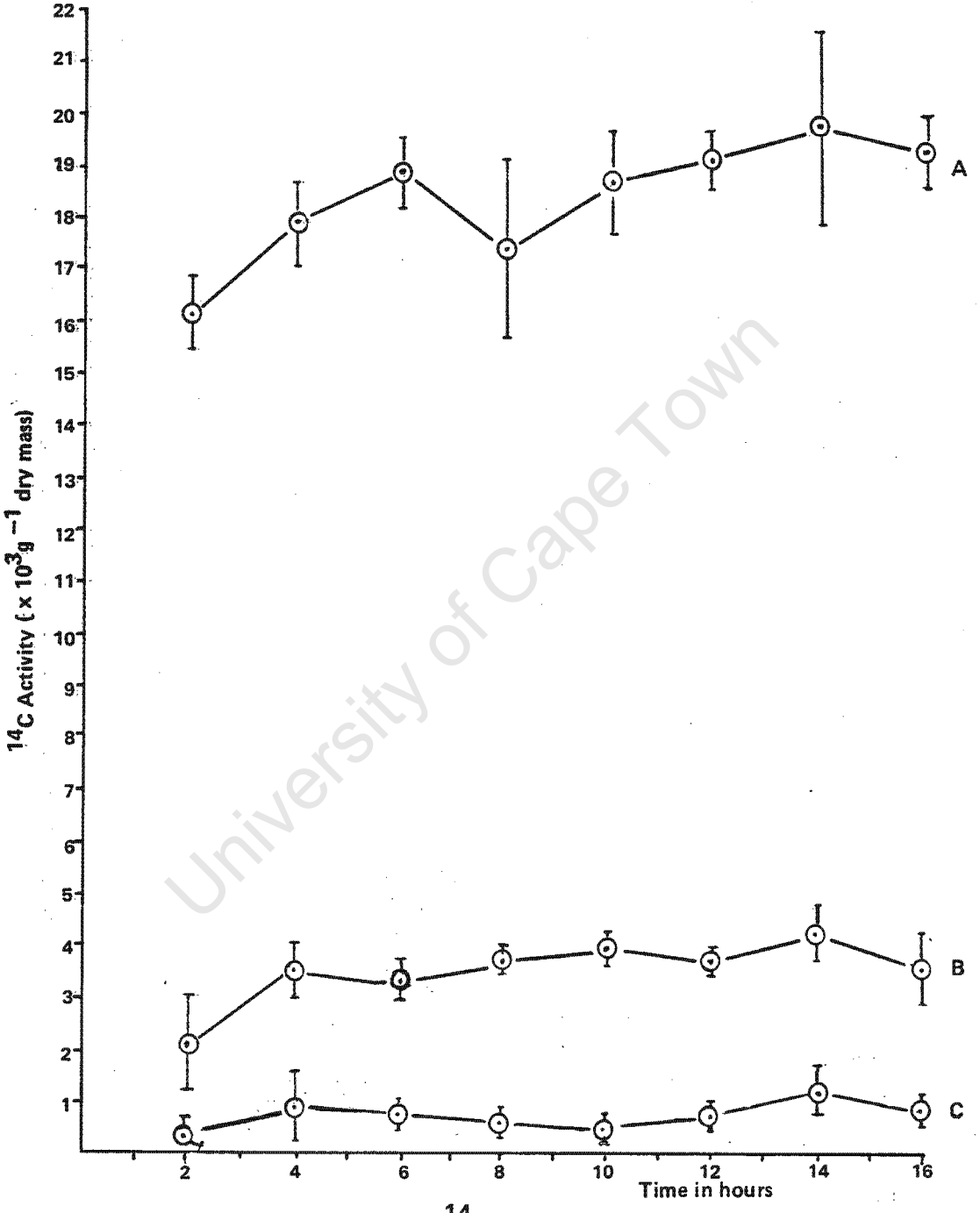


FIG.25 A time course of the ^{14}C-labelled assimilates after incubation in NaH$^{14}CO_3$ seawater in *S. vittata*.

A = *S. vittata*

B = combined samples of whole plants of *S. vittata* and the portions of stipe of *E. maxima* to which they were attached

C = stipe of *E. maxima* with no attached red algae

Results are means of six replicates and vertical bars represent twice the SEM.

TABLE 10

The percentage distribution of recovered ^{14}C-activity in the ethanol-soluble, starch-containing and acid-hydrolysed fractions of *C. minima* and *S. vittata* after incubation in ^{14}C-mannitol (0,5 µCi $m\ell^{-1}$) for four, eight and twenty four hours. Results are means of three samples taken from three separate experiments.

Carpoblepharis minima

Time in hours	Percentage Distribution			
	Ethanol-soluble	Starch-containing	Acid-hydrolysate	Total activity
4	*84,0 (66,2 ± 1,7)	* 8,0 (16,4 ± 0,8)	* 7,0 (15,3 ± 0,8)	° 16 ± 1,4
8	*85,0 (67,2 ± 1,5)	* 5,0 (12,9 ± 0,8)	*10,0 (18,4 ± 1,1)	° 29 ± 2,7
24	*86,0 (68,0 ± 1,8)	* 5,0 (12,9 ± 1,0)	*10,0 (18,4 ± 1,0)	° 31 ± 2,7

Suhria vittata

Time in hours	Percentage Distribution			
	Ethanol-soluble	Starch-containing	Acid-hydrolysate	Total activity
4	*80,0 (63,4 ± 1,2)	*12,0 (20,3 ± 1,0)	*10,0 (18,4 ± 1,0)	° 4 ± 1,6
8	*40,0 (39,2 ± 1,4)	*45,0 (42,1 ± 0,9)	*15,0 (22,8 ± 1,3)	° 5 ± 1,4
24	*38,0 (38,1 ± 1,3)	*49,0 (44,4 ± 1,1)	*12,0 (20,3 ± 1,2)	° 8 ± 2,7

* Percent of total with angular transformation ± SEM in parentheses

° Total activity (DPM x $10^3 g^{-1}$ dry mass ± SEM)

although 80% of the ^{14}C-label was in the ethanol-soluble fraction after four hours, it dropped to 40% after eight hours and 38% after twenty four hours, while the ^{14}C-label in the starch containing fraction over the same intervals of time, rose from 12% to 45% to 49% (Table 10). This suggested that the assimilated mannitol in *S. vittata* was converted to a compound, probably starch, which was hydrolysed by the enzyme α-amylase.

4.3 Photosynthetic Pigments

The photosynthetic pigments scanned between 390 and 680nm are shown in Fig. 26 for *C. minima* and Fig. 27 for *S. vittata*. These results were compared with the known absorption peaks of various algal pigments (Govindjee and Braun, 1974). Both algae contained chlorophyll a which occurs in all algae and is the main photosynthetic pigment in all oxygen evolving photosynthetic organisms, although it exists in different forms which absorb at different wavelengths (Govindjee and Braun, 1974). These red algae also possessed α-and β-carotene which transfer energy to chlorophyll *a* and the water soluble pigments, phycoerythrins, R and B phycoerythrin which are found in the Rhodophyceae are responsible for the red colour in this class.

4.4 Identification of the Major Carbohydrates

The total sugars in an ethanol-soluble fraction were analysed by the phenol/sulphuric acid method (Dubois *et al.*, 1956). The results for *C. minima* and *S. vittata* are given in Table 11 and show that *S. vittata* had a higher concentration of sugars in an ethanol-soluble fraction than *C. minima*. Chromatograms and GLC were used to separate and identify these carbohydrates. A typical paper chromatogram of the ethanol-soluble carbohydrates is given in Pl. 6A for *C. minima* and Pl. 6B for *S. vittata*. The carbohydrate present was mannitol which had an Rg value of 1,19 ± 0,001 in both red algae. Typical GLC traces of the ethanol-soluble carbohydrates are given for both red algae in Fig. 28 and the acid-hydrolysed fraction in Fig. 29. The quantitative data, given on the figures, of the concentration of various components of the fractions are the mean results

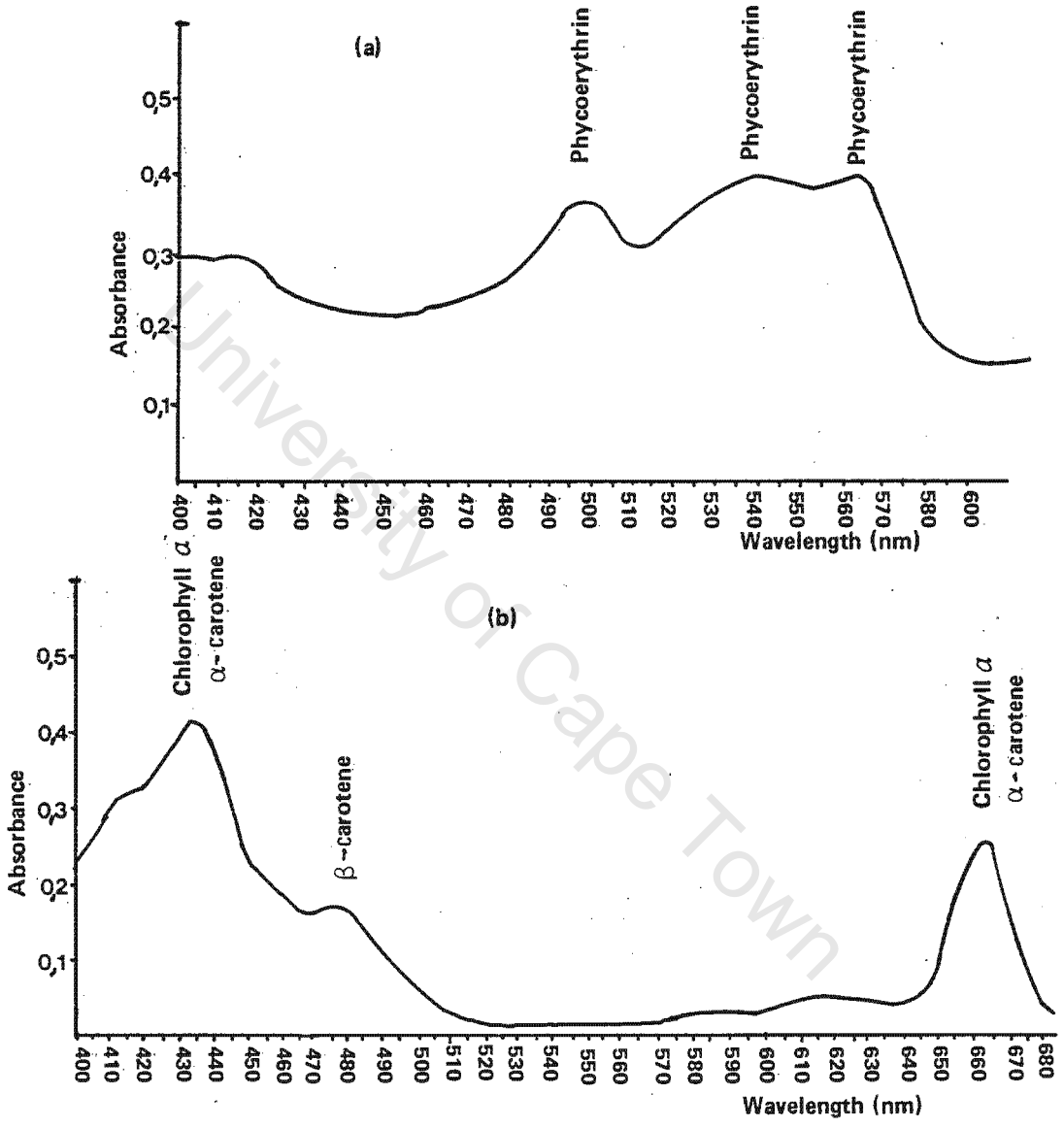


FIG. 26 The photosynthetic pigments present in (a) aqueous and (b) petroleum-ether extracts of *C. minima.*

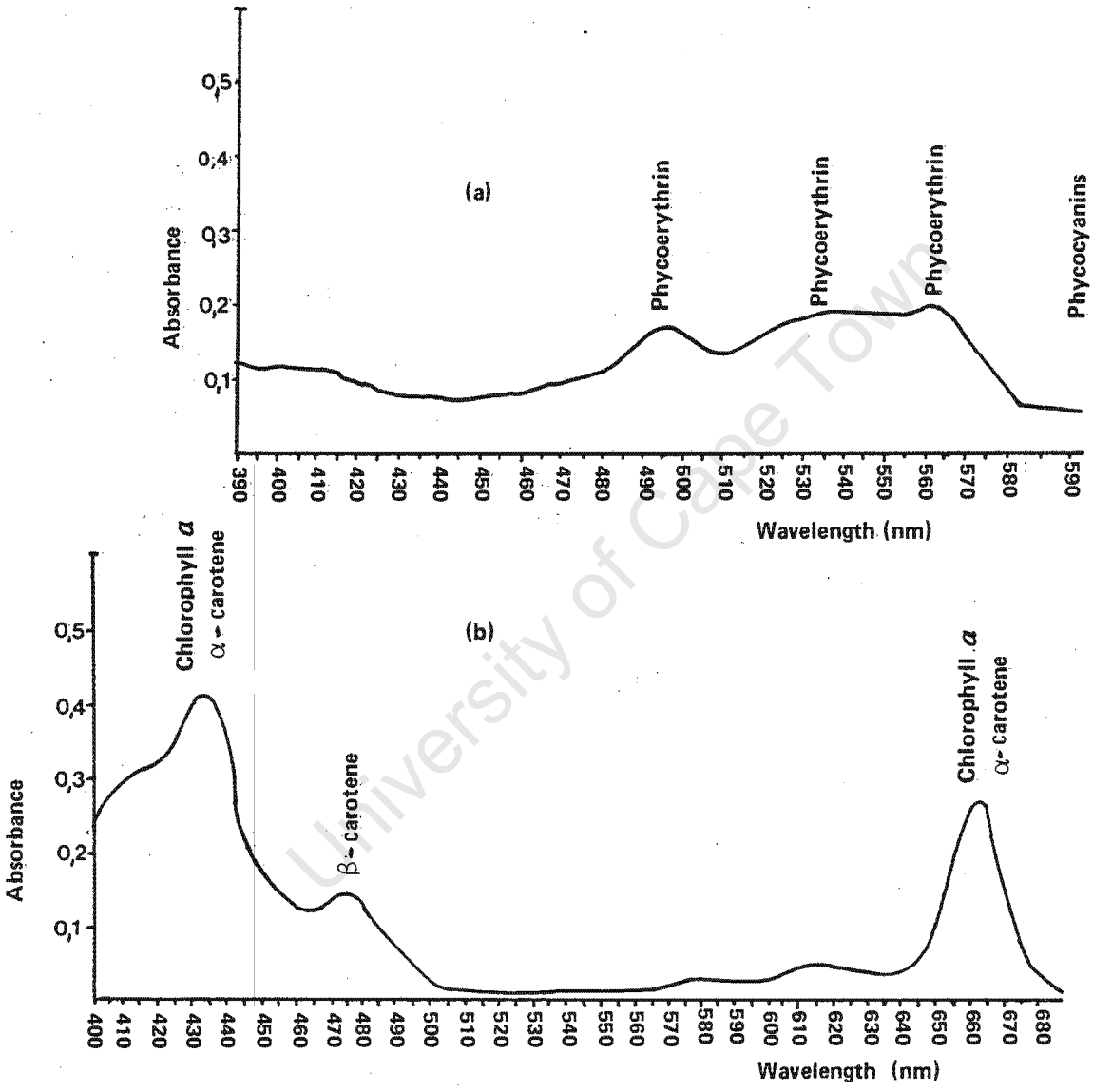


FIG.27 The photosynthetic pigments present in (a) aqueous and (b) petroleum ether extracts of *S. vittata*.

TABLE 11

The total carbohydrates of an ethanol-soluble fraction of *C. minima* and *S. vittata*, deproteinized, deionized and calculated as glucose units using the method of Dubois *et al.* (1956). Each figure is a mean of three samples taken monthly from August to December 1979. There was no significant difference at the 5% level between the results.

Algae	Total carbohydrates in mg g^{-1} dry mass $\pm$ SEM
C. minima	79 $\pm$ 1,69
S. vittata	85 $\pm$ 1,07

PLATE 6

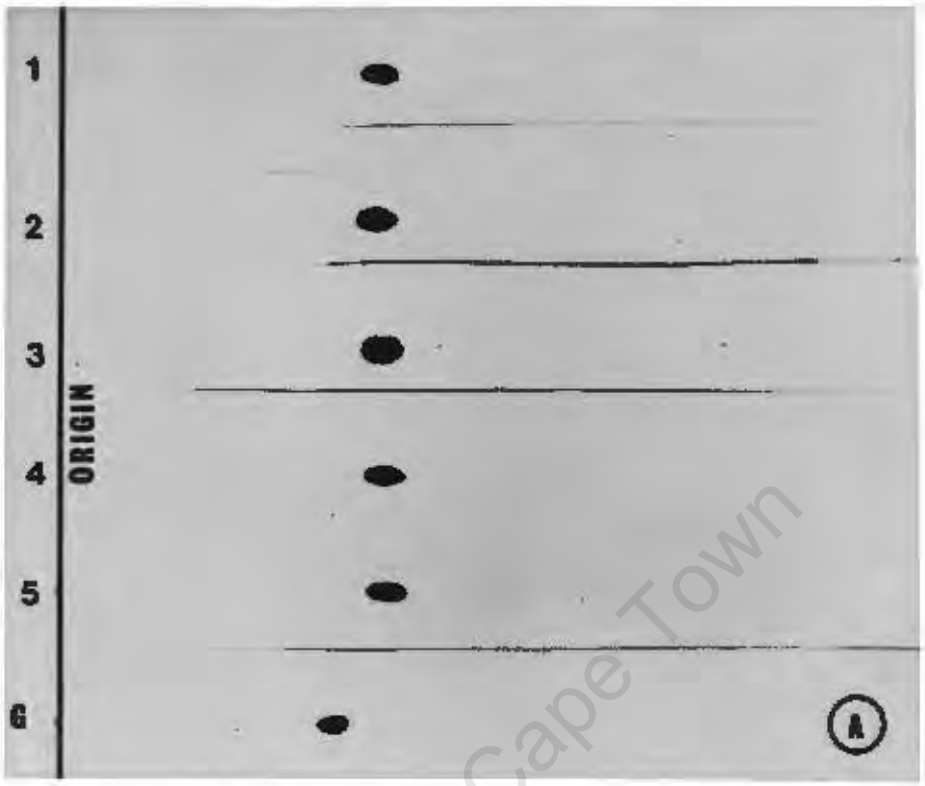
A

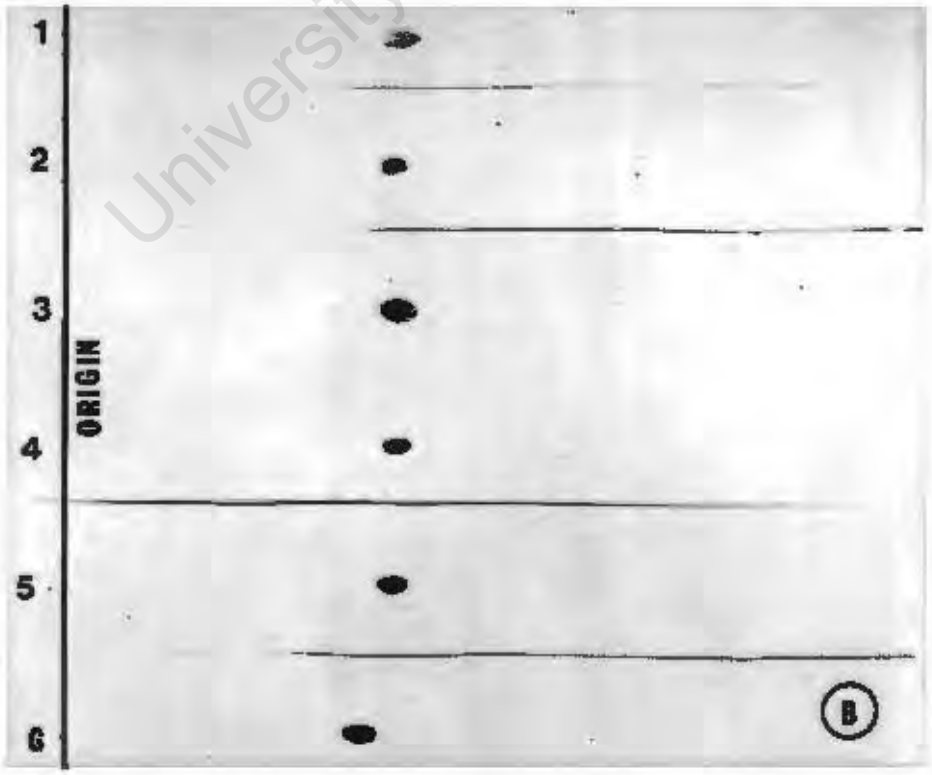
A paper chromatogram of an ethanol-soluble extract of *C. minima* run for forty one hours at 20-22°C in ethyl acetate:acetic acid:water (14:3:3) by means of descending chromatography. Carbohydrate spots were detected by the silver nitrate/sodium ethoxide method of Trevelyan *et al.* (1950). The origin is marked, numbers 1-5 are replicates of *C. minima* and G is the standard glucose marker

B

A paper chromatogram of an ethanol-soluble extract of *S. vittata* run for forty one hours at 20-22°C in ethyl acetate:acetic acid:water (14:3:3) by means of descending chromatography. Carbohydrate spots were detected by the silver nitrate/sodium ethoxide method of Trevelyan *et al.* (1950). The origin is marked, numbers 1-5 are replicates of *S. vittata* and G is the standard glucose marker

PLATE 6





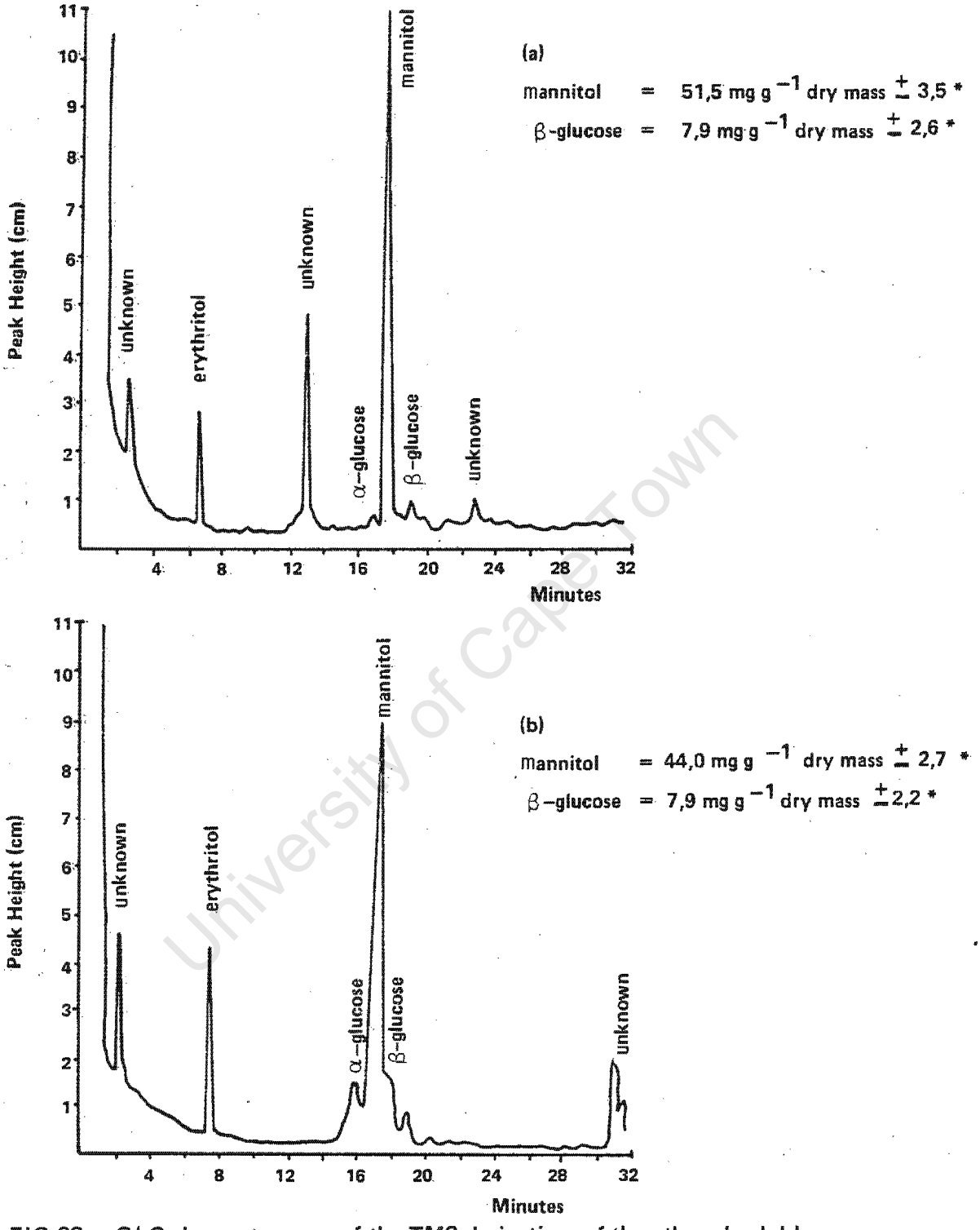


FIG.28 GLC chromatograms of the TMS derivatives of the ethanol soluble fraction of the whole plant of (a) *C. minima* and (b) *S. vittata*. Erythritol was used as an internal standard. * Each is a mean of 3 values ± SEM.

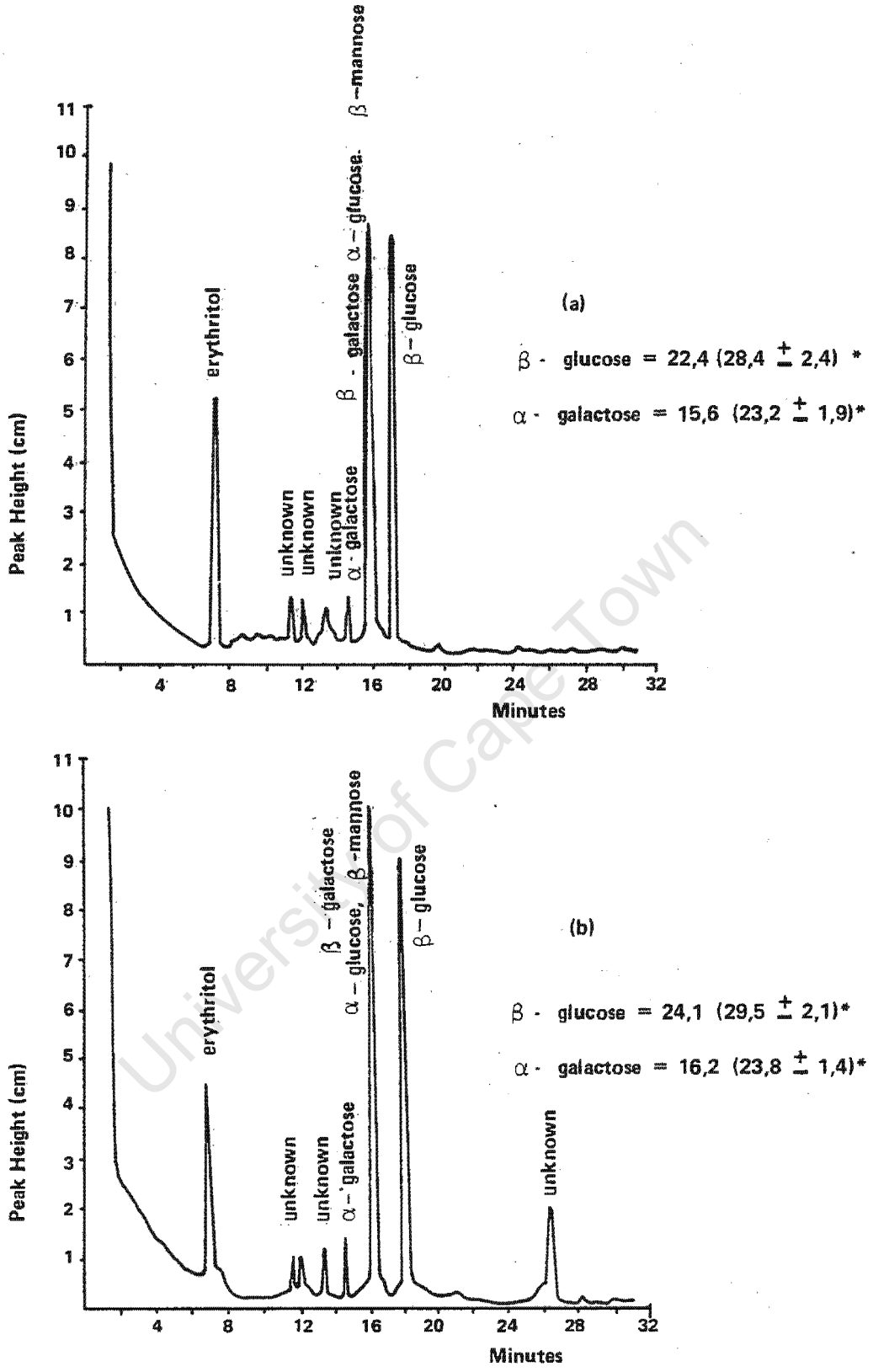


FIG.29 GLC Chromatograms of the TMS derivatives of the acid hydrolysed fraction of the whole plant of (a) *C. minima* and (b) *S. vittata*. Erythritol was used as an internal standard. * Expressed as mol - %, angular transformations of percentages are shown in brackets ± SEM. Each is a mean of 3 values.

of three different GLC traces. Mannitol was the major component of the ethanol-soluble carbohydrates in both red algae with a higher concentration (71,5mg g^{-1} dry mass) in *C. minima* than in *S. vittata* (44,0mg g^{-1} dry mass). The acid hydrolysed fraction showed two major components in both red algae (Fig. 29). Each sugar component was expressed as a mol% of the total molar proportion of the fraction. Figures in brackets are the angular transformation ± SEM. In *C. minima* there was 22,4% (28,3 ± 2,4) β-glucose and 15,6% (23,2 ± 1,9) α-galactose. In *S. vittata* there was 24,1% (29,5 ± 2,1) β-glucose and 16,2% (23,8 ± 1,4) α-galactose. Various unidentified compounds were found in both red algae. The ethanol-soluble fractions of the unknowns had retention times of 2,5, 12,8 and 22,9 minutes in *C. minima* and 2,0 and 30,8 minutes in *S. vittata* (Fig. 28). The retention times in the acid-hydrolysed fractions of the unknowns were 11,4, 12,0 and 13,4 minutes in *C. minima* and 11,5, 12,0 and 26,5 minutes in *S. vittata*.

4.5 Analysis of "Free" Amino Compounds

The "free" amino acids present in *C. minima* and *S. vittata* are given in Table 12. The major "free" amino acid in *C. minima* was alanine followed by glutamine and glutamic acid, whereas in *S. vittata* it was glutamine followed by glutamic acid and glycine.

4.6 Discussion

The results presented in this chapter have demonstrated that both red algae were capable of assimilating by photosynthesis ^{14}C-label both in the form of $NaH^{14}CO_3$ and ^{14}C-mannitol when these were present in the seawater surrounding the plants. An analysis of the petroleum ether and aqueous extracts of pigments of *C. minima* (Fig. 26) and *S. vittata* (Fig. 27) demonstrated that they possessed all the photosynthetic pigments necessary for an autotrophic existence and their ability, therefore, to fix the ^{14}C-label supplied in seawater was not surprising. In a review article on red algal parasites (Evans *et al.*, 1978) it was shown that at least half were white, lacking pigmentation, or pink,

TABLE 12

Concentration of "free" amino acids in μmoles g^{-1} fresh mass in *C. minima* and *S. vittata*.

Amino Acid	*C. minima*	*S. vittata*
Aspartic acid	0,194	0,097
Threonine	0,052	0,063
Serine	0,159	0,142
Asparagine	0,024	0,028
Glutamic acid	0,274	0,662
Glutamine	0,299	0,743
Glycine	0,060	0,164
Alanine	0,539	0,156
Valine	0,036	0,014
Cystine	0,009	0,076
Methionine	0,019	0,003
Isoleucine	0,011	0,007
Leucine	0,018	0,009
Tyrosine	0,007	0,004
Phenylalanine	0,054	0,147
Lysine	0,039	0,016
Histidine	0,281	0,030

with slight pigmentation. Several of the species dealt with had either reduced or no plastids present. Goff (1976) found the parasite *Harveyella* to be colourless and this lack of pigmentation seems to be fairly common in parasitic red algae. The red algae in *C. minima* and *S. vittata* showed the typical red colour of the Rhodophyceae and possessed the pigments chlorophyll, phycobolin and carotene (Fig. 26 and Fig. 27).

The majority of the ^{14}C-label was incorporated into the ethanol-soluble fraction in both red algae after being incubated in exogenously supplied ^{14}C-labelled mannitol (Table 10). The percentage ^{14}C-label in each fraction of *C. minima* (Table 10) remained relatively constant over the time span of the experiment with the majority in the ethanol-soluble fraction. The greatest percentage of recovered ^{14}C-label in *S. vittata* was in the ethanol-soluble fraction but this decreased during the course of the experiment while the percentage recovered from the starch-containing fraction increased. *Suhria vittata* took up about 75% less ^{14}C-label from exogenously supplied mannitol than *C. minima*. Craigie (1974) stated that the Ceramiales, the class to which *C. minima* belongs, were the only red algae to accumulate mannitol, which being an acyclic polyol is ethanol-soluble. Paper chromatography and GLC showed the major carbohydrate of the ethanol-soluble fraction to be mannitol in both red algae. Craigie (1974) has reported the presence of mannitol in sixteen species of red algae including *Polysiphonia lanosa*. Evans *et al.* (1978) found sodium mannoglutarate to be the major product of photosynthesis in *P. lanosa*, this compound being restricted to the Ceramiales. Lewis and Smith (1967a) in a review of the distribution of polyols in algae, have reported the presence of mannitol in twelve species of red algae and the polyols dulcitol and sorbitol in *Bostrychia scorpiodes* and mannitol and volemitol in *Porphyra umbilicalis*. Majak *et al.* (1966) found mannitol was produced during photosynthesis of $^{14}CO_2$ by nine out of twelve species studied, but in six of the species small amounts of ^{14}C-label were found in mannitol, far less than

incorporated into floridoside which may be one of the reasons earlier authors failed to detect it. Majak *et al.* (1966) concluded that mannitol should be considered a normal, but not abundant, metabolite of red algae. Kremer (1976a) retested species of red algae in which mannitol had been reported, including freshwater species, but unlike *C. minima* and *S. vittata*, these red algae did not grow in close association with brown algae. Mannitol, or any other hexitol, could not be detected in any of the species, either as a ^{14}C-assimilate after photosynthetic assimilation of ^{14}C from $H^{14}CO_3^-$, or in trace amounts in the ethanol-soluble fraction. Attempts to qualify the action of a specific mannitol synthesizing enzyme (mannitol-1-phosphate dehydrogenase, E.C.1.1.1.17) also proved negative. Kremer (1976a) suggested that the presence of mannitol reported in the red algae may have been due to incorrect identification, mannose heteroside having been identified as mannitol, especially in the Ceramiales which contain mannoglycerate. Kremer (1976b) reported the presence and metabolism of dulcitol and sorbitol in the marine red alga *Bostrychia scorpiodes* and the presence of floridoside as a main accumulation product of photosynthesis in some species, while other species, including members of the Ceramiales, incorporate the ^{14}C-label in mannosidoglycerate (Kremer, 1978a). Exogenously supplied mannitol was taken up from seawater but *Porphyra umbilicalis*, *Chondrus crispus* and *Rhodomela confervoides* removed not more than 1% of the total amount of ^{14}C-labelled mannitol supplied even after three hours. *Carpoblepharis minima* was found to have removed 17% of the total ^{14}C-activity and *S. vittata* 4% of the total ^{14}C-activity due to exogenously supplied ^{14}C-mannitol. The incorporation of ^{14}C-mannitol (Table 10) and $NaH^{14}CO_3$ (Fig. 24 and Fig. 25) by *C. minima* and *S. vittata* showed that they were capable of photosynthesis. When the ^{14}C-label was in the form of $H^{14}CO_3^-$ *S. vittata* showed a greater activity, due to the presence of ^{14}C-label in the tissues of the plant, than *C. minima* (Fig. 24 and Fig. 25).

In *L. pallida* and *E. maxima*, in addition to mannitol, after incorporation of the ^{14}C-label by photosynthesis, the other

compounds identified which carried the ^{14}C-label were certain amino acids (Chapter 3). It was, therefore, important to identify the "free" amino acids of the red algae in order to establish if they were identical to those in the brown algae as this would mean that the red algae could metabolise those amino acids supplied by the brown algae. The major "free" amino acids in *C. minima* were alanine, glutamine and glutamic acid (Table 12) whereas those in *L. pallida* were alanine, glutamic acid, glutamine, aspartate and histidine (Chapter 3). The major "free" amino acids in *S. vittata* were glutamine, glutamic acid and glycine (Table 12) and in *E. maxima* were alanine, glutamic acid, glutamine and aspartate (Chapter 3). *Carpoblepharis minima* and *S. vittata*, therefore, contained the same major "free" amino acids as their associated brown algae.

CHAPTER 5

PHYSIOLOGICAL STUDIES ON THE *LAMINARIA PALLIDA/CARPOBLEPHARIS MINIMA* AND *ECKLONIA MAXIMA/SUHRIA VITTATA* ASSOCIATIONS

5.1 Introduction

The studies in Chapters 3 and 4 form a background for the investigation of the red/brown algal relationship. The emphasis was on physiological and biochemical studies as pigmentation and morphology have been shown to be unreliable criteria (Evans *et al.*, 1978).

The problem in studying any plant association lies in the terminology which should be followed, although the terms epiphyte (plants using other plants purely for mechanical support) and parasite (plants totally dependent on other organisms for their nutrition) have been clearly defined (Andrews, 1976; Evans *et al.*, 1978; Lewis, 1974). Plants which are dependent for some, but not all, of their nutrition on another plant have a series of names, hemi-parasites, holoparasites and partial parasites. Lewis (1974) in a review on the evolution of parasitism and mutualism has proposed a classification which would be applicable to the majority of relationships of micro-organisms. None of these terms is very satisfactory when applied to the *L. pallida/C. minima* and *E. maxima/S. vittata* associations. It is difficult to establish the exact degree of dependence of a plant on a host to which it grows attached when it is capable of assimilating some of its own nutrients. An association which lies somewhere between a "true epiphyte" and a "true parasite" has not been adequately defined. *Carpoblepharis minima* and *S. vittata* have been termed epiphytes (Simons, 1976). This study will establish if there is some transfer of the assimilates of photosynthesis from brown algal host to the red algal partner.

5.2 Translocation of ^{14}C-labelled Assimilates from the Brown to the Red Algae

Using the perspex chambers (Fig. 8, Chapter 3) only the frond was incubated with ^{14}C-label and the distribution of ^{14}C-assimlates in the brown and the red algae attached to the brown algal stipe is given in Table 13. Any red algae attached to the frond were not analysed as these were exposed directly to the ^{14}C-label. In those sporophylls chosen for experimentation, which had red algae attached to the stipe, it was necessary to have at least six or more individual red algal plants present to allow a large enough sample for experimentation. Results are given as a percentage of the total activity in the whole plant recovered in each region. There was a greater movement of ^{14}C-label out of the frond of *E. maxima* than from *L. pallida* (Table 13). In *L. pallida*, however, the majority of translocated ^{14}C-label was found in the attached red algae (8,6%) with a smaller percentage present in the stipe; whereas in *E. maxima* the majority of ^{14}C-label was in the stipe with only 0,3% in the attached red algae. The percentage recovered ^{14}C-label in *C. minima* was probably due to active movement of the assimilates of photosynthesis from the host, but in *S. vittata* it was so low due to a passive process. Analysis of the seawater around the stipe, together with other methods described in Chapter 3, were also carried out in these experiments to ensure that no leakage of ^{14}C-label occurred across the seal between the two chambers (Fig. 8, Chapter 3). Comparison of the results in Table 1 (Chapter 3) (brown algae with no attached red algae) with the results in Table 13 (kelp with attached red algae) reveals a major difference between *C. minima* and *S. vittata*. In the kelp with no attached red algae (Table 1, Chapter 3) the percentage distribution of activity was 12,5% in the stipe and 1,8% in the holdfast of *L. pallida* and 18,2% in the stipe and 2% in the holdfast of *E. maxima*. When red algae are attached to the brown algae a slightly different distribution of ^{14}C-label is obtained in the *L. pallida/C. minima* association but not in that of *E. maxima/S. vittata*. Table 13 shows that 8,6% of the incorporated ^{14}C-label in *L. pallida/C. minima* was present in the red algae with the percentage in the stipe

TABLE 13

The percentage distribution of recovered radioactivity in the stipe of *L. pallida/C. minima* and the stipe of *E. maxima/ S. vittata* associations after six hours incorporation. Only the frond of the brown algae was exposed to ^{14}C-bicarbonate ($1\mu Ci\ m\ell^{-1}$). Results are means of thirty six replicates taken from six plants.

Region	*L. pallida/C. minima*	*E. maxima/S. vittata*
* Incubated Frond	86,3 (68,3 ± 1,9)	77,7 (61,9 ± 2,0)
* Stipe	3,7 (11,1 ± 0,4)	20,7 (27,1 ± 0,8)
* Holdfast	1,4 (6,7 ± 0,7)	1,3 (6,5 ± 1,2)
* Red algae attached to stipe	8,6 (17,0 ± 0,8)	0,3 (3,0 ± 0,6)
* Percent exported	13,7 (21,7 ± 1,0)	22,3 (28,3 ± 1,3)
° Total activity	29,0 ± 3,1	44,0 ± 3,7

* Percent of total with angular translocation ± SEM in parentheses

° Total activity (DPM x $10^3 g^{-1}$ dry mass ± SEM)

having dropped to 3,7%, whereas in *E. maxima* only 0,3% was present in *S. vittata* and 20,7% remaining in the stipe. In both brown algae the presence of red algae caused a lower percentage of the ^{14}C-label to be found in the holdfast dropping from 1,8% to 1,4% in *L. pallida* and from 2,0% to 1,3% in *E. maxima* (Table 1 and Table 13). The presence of the red algae, therefore, appeared to affect slightly the normal distribution of the assimilates of photosynthesis.

5.3 The Degree of Colonization of *C. minima* on *L. pallida* and *S. vittata* upon *E. maxima*

The degree of colonization of red algae upon one metre high plants of *L. pallida* and *E. maxima* was found to be dependent on the time of the year. The results for the number of *C. minima* plants per $50cm^2$ of stipe of *L. pallida* are given in Fig. 30 (Appendix Table 12). A similar colonization study of *S. vittata* upon *E. maxima* was not carried out because *E. maxima* has been commonly found to be infested by another red alga, *Carpoblepharis flaccida* (Turn.) Kutz. In this study, portions of stipe of whole plants of *E. maxima* on which only *S. vittata* was present, had to be selected. It was decided that such plants would not yield a representative picture of the degree of colonization by *S. vittata* as *in vivo* it is normally in competition with *C. flaccida*. It was noticed, in general, that *E. maxima* had a greater number of red algae attached to it than *L. pallida*. In both kelps the stipe was colonized before the frond. Red algae were rarely found on young actively growing plants (smaller than 1m in length) but on larger, older plants (usually over 2m) which had extreme frond erosion due to winter storms, there was heavy colonization. The results in Fig. 30 for the stipe of *L. pallida* agree with these observations, with heaviest colonization occurring in April and May (autumn/early winter). The colonization intensity decreased by June, July and August and was presumably due to these plants being eroded by the winter storms which are particularly severe in the South-western Cape waters. Active growth in the kelp is during spring and summer, August to December (Dieckmann, 1978; Mann *et al.*, 1979).

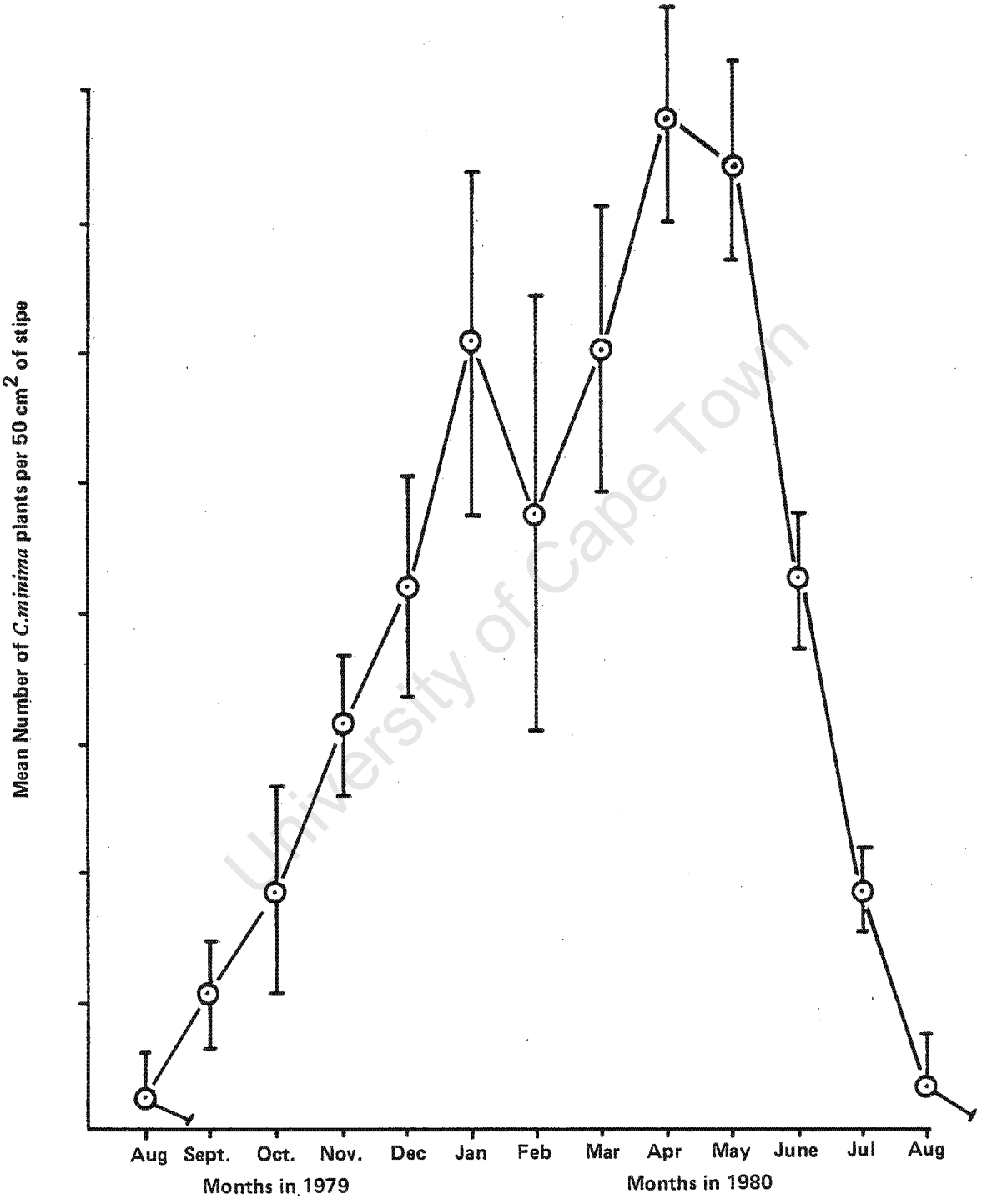


FIG.30 The number of *C. minima* plants growing attached to 50 cm^2 of stipe ± SEM of about 1 m plants of *L. pallida* over 13 months.

A series of experiments was carried out using about one metre plants with a varying number of red algae upon the stipe to determine whether this affected the movement of ^{14}C-label from the frond and its distribution in the different regions of the plants, namely the stipe and holdfast of the brown algae and the attached red algae. As previously mentioned, no plants with fewer than six red algae on the stipe were analysed. The plants were incubated in the chambers described in Chapter 3 (Fig. 8) where only the frond was exposed to $NaH^{14}CO_3$ in seawater and plants were analysed in an identical manner to that described in section 3.2. The results of the distribution of ^{14}C-label in plants with different numbers of attached red algae are shown in Tables 14 and 15. The distribution of ^{14}C-label in the ethanol-soluble, starch containing and acid-hydrolysed fractions after a time course study, is presented in Tables 16 and 17. The presence of larger numbers of *C. minima* plants caused a greater percentage of the recovered ^{14}C-label to accumulate in the red algae (Table 14). A larger number of red algae attached to the stipe caused a greater flow of assimilates of photosynthesis from the frond where they are formed via the stipe to the red algae (Table 14). There was no discernible pattern in either the percentage recovered ^{14}C-label in *S. vittata* or the amount of ^{14}C-label exported as the number of *S. vittata* plants present on the stipe of *E. maxima* increased. The amount of ^{14}C-label in *S. vittata* ranged from 0,3 to 0,5% (Table 15). There was transfer from the frond of *E. maxima* to the stipe with the percent recovered ^{14}C-label ranging from 19,3 to 22,2% but did not show any pattern due to the presence of red algae (Table 15). In both brown algae the amount of ^{14}C-label present in the holdfast was minimal, 0,6 to 1,0% (Table 14 and Table 15).

The distribution of ^{14}C-label in the various fractions (Table 16 and Table 17) showed that the $NaH^{14}CO_3$ in seawater taken up by the frond during photosynthesis, was translocated to the stipe. In the *L. pallida/C. minima* association it was transferred to *C. minima* (Table 16) but not to *S. vittata* from the stipe of *E. maxima* (Table 17). When samples of

TABLE 14

The percentage distribution of recovered radioactivity in *L. pallida/C. minima* with varying numbers of red algae upon the stipe. Experimental procedure was identical to that used to obtain the results in Tables 1 and 13.

Region	Total number of red algal plants on the stipe							
	6	10	12	17	25	31	40	56
* Frond	87,4 (69,2 ± 1,0)	85,2 (67,3 ± 1,3)	86,1 (68,1 ± 1,7)	83,2 (65,7 ± 0,6)	82,2 (65,0 ± 1,2)	80,0 (63,4 ± 1,0)	77,9 (62,0 ± 1,8)	74,6 (59,8 ± 1,5)
* Stipe	5,0 (12,9 ± 0,7)	6,0 (14,2 ± 0,8)	5,5 (13,5 ± 0,6)	7,4 (15,8 ± 0,7)	8,2 (16,6 ± 0,8)	9,1 (17,5 ± 0,9)	10,7 (19,1 ± 1,1)	13,4 (21,4 ± 0,9)
* Holdfast	0,9 (5,3 ± 0,6)	0,8 (5,0 ± 0,7)	1,0 (5,7 ± 0,7)	0,9 (5,3 ± 0,6)	0,7 (4,7 ± 0,9)	0,6 (4,4 ± 0,8)	0,6 (4,4 ± 1,0)	0,6 (4,4 ± 0,8)
* Red algae	6,7 (15,0 ± 0,7)	8,0 (16,4 ± 0,6)	7,4 (15,8 ± 0,6)	8,5 (16,9 ± 1,0)	8,9 (17,3 ± 0,8)	10,3 (18,7 ± 1,1)	10,8 (19,2 ± 0,9)	11,4 (19,7 ± 1,2)
* Percent exported	12,6 (20,8 ± 1,3)	14,8 (22,6 ± 1,2)	13,9 (21,9 ± 0,7)	16,8 (24,1 ± 1,0)	17,8 (25,0 ± 0,9)	20,0 (26,6 ± 1,4)	22,1 (28,1 ± 1,1)	25,4 (30,3 ± 1,0)
° Total activity	32,5 ± 1,5	32,2 ± 1,9	31,6 ± 2,0	30,7 ± 1,8	36,1 ± 2,0	38,8 ± 2,3	40,6 ± 2,9	43,8 ± 2,4

* Percent of total with angular transformation ± SEM in parentheses

° Total activity (DPM x $10^3 g^{-1}$ dry mass ± SEM)

TABLE 15

The percentage distribution of recovered radioactivity in *E. maxima/S. vittata* with varying numbers of red algae upon the stipe. Experimental procedure was identical to that used to obtain the results in Tables 1 and 13.

Region	Total number of red algal plants on the stipe							
	6	11	16	20	25	34	40	55
* Frond	79,4 (63,0 ± 1,6)	78,7 (62,5 ± 1,3)	78,6 (62,5 ± 1,0)	79,2 (62,9 ± 1,1)	76,5 (61,0 ± 0,9)	77,3 (61,6 ± 1,7)	78,2 (62,2 ± 1,5)	77,6 (61,8 ± 1,2)
* Stipe	19,3 (26,1 ± 0,9)	20,2 (26,8 ± 1,3)	20,5 (27,0 ± 1,0)	19,7 (26,4 ± 0,8)	22,2 (28,2 ± 1,1)	21,7 (27,9 ± 1,4)	20,9 (27,3 ± 0,8)	21,3 (27,6 ± 1,0)
* Holdfast	1,0 (5,7 ± 0,7)	0,8 (5,0 ± 0,8)	0,6 (4,4 ± 1,0)	0,7 (4,7 ± 0,8)	0,8 (5,0 ± 0,7)	0,6 (4,4 ± 0,7)	0,6 (4,4 ± 1,0)	0,7 (4,7 ± 0,9)
* Red algae	0,3 (3,0 ± 0,6)	0,3 (3,0 ± 0,6)	0,3 (3,0 ± 0,7)	0,4 (3,5 ± 0,6)	0,5 (4,0 ± 0,8)	0,4 (3,5 ± 0,6)	0,3 (3,0 ± 0,6)	0,4 (3,5 ± 0,6)
* Percent exported	20,6 (27,0 ± 1,2)	21,3 (27,5 ± 0,9)	21,4 (27,6 ± 0,8)	20,8 (27,1 ± 1,2)	23,5 (29,1 ± 1,4)	22,7 (18,5 ± 0,8)	21,8 (27,9 ± 1,0)	22,4 (28,3 ± 0,9)
° Total activity	43,2 ± 2,3	46,5 ± 3,0	49,4 ± 2,7	44,0 ± 1,9	48,2 ± 2,6	44,3 ± 2,5	50,5 ± 2,8	50,9 ± 3,1

* Percent of total angular transformation ± SEM in parentheses

° Total activity (DPM x $10^3 g^{-1}$ dry mass ± SEM)

TABLE 16

The percentage distribution of radioactivity in the ethanol-soluble, starch-containing and acid-hydrolysed fractions of pooled samples of brown algal stipe and attached red algae. Plants were incubated in the chambers shown in Fig. 8 with only the frond in contact with the ^{14}C-label ($1\mu Ci\ m\ell^{-1}$) Samples were taken after four, eight and twenty four hours. Results are means of twenty four replicates (six samples from four separate experiments).

Algal Fraction	*L. pallida/C. minima*			*E. maxima/S. vittata*		
	4h	8h	24h	4h	8h	24h
* Ethanol-soluble	87,0 (68,9 ± 1,8)	85,0 (67,2 ± 1,6)	84,0 (66,4 ± 1,8)	79,0 (62,7 ± 1,5)	74,0 (58,3 ± 1,7)	69,0 (56,2 ± 1,2)
* Starch-containing	7,0 (15,3 ± 0,8)	6,0 (14,2 ± 1,0)	9,0 (15,3 ± 0,8)	10,0 (18,4 ± 1,1)	16,0 (23,6 ± 0,7)	18,0 (25,1 ± 0,8)
* Acid hydrolysate	6,0 (14,2 ± 0,9)	9,0 (17,5 ± 0,7)	7,0 (15,3 ± 1,0)	11,0 (19,4 ± 1,0)	10,0 (18,4 ± 1,2)	13,0 (21,1 ± 0,9)
° Total activity	23,0 ± 3,4	31,7 ± 4,6	38,2 ± 3,5	22,1 ± 1,8	25,7 ± 2,2	29,4 ± 2,0

* Percent of total with angular transformation ± SEM in parentheses

° Total activity (DPM x $10^3 g^{-1}$ dry mass ± SEM)

TABLE 17

The percentage distribution of radioactivity in the ethanol-soluble, starch-containing and acid-hydrolysed fractions of samples of red algae. Plants were incubated in the chambers shown in Fig. 8 with only the frond in contact with the ^{14}C-label (1µCi $m\ell^{-1}$). Samples were taken after four, eight and twenty four hours. Results are means of twenty four replicates (six samples from four separate experiments).

Algal Fraction	*C. minima*			*S. vittata*		
	4h	8h	24h	4h	8h	24h
* Ethanol-soluble	86,0 (68,0 ± 1,9)	80,0 (63,4 ± 2,3)	78,0 (62,0 ± 1,4)	NA	NA	NA
* Starch-containing	5,0 (12,9 ± 1,0)	7,0 (15,3 ± 0,9)	9,0 (17,5 ± 0,6)	NA	NA	NA
* Acid hydrolysate	9,0 (17,5 ± 0,7)	13,0 (21,5 ± 1,3)	13,0 (21,1 ± 0,9)	NA	NA	NA
° Total activity	24,3 ± 1,1	27,1 ± 1,9	32,0 ± 2,7	NA	NA	NA

* Percent of total with angular transformation ± SEM in parentheses

° Total activity (DPM x $10^3 g^{-1}$ dry mass ± SEM)

NA = no activity i.e., no ^{14}C-label recovered

E. maxima and *S. vittata* were pooled ^{14}C-label was recovered but samples of *S. vittata* alone showed no radioactivity. Thus, all the ^{14}C-label recovered in the former samples came from the *E. maxima* stipe. In the *L. pallida/C. minima* relationship, ^{14}C-label was found in pooled samples of brown algal stipe and red algae (Table 16) as well as in the samples of red algae alone (Table 17). The majority of ^{14}C-label, in all cases, was found in the ethanol-soluble fraction and diminished over the twenty four hours of the experiment. The percentage in the starch-containing fraction increased over the time period from 7,0 to 9,0% in the *L. pallida/C. minima* association and from 10,0 to 18,0% in the *E. maxima/S. vittata* association (Table 16). *Carpoblepharis minima* alone showed similar trends to pooled samples of red and brown algae and in all samples the percentage recovered ^{14}C-label in the acid hydrolysate fraction remained relatively constant. *Carpoblepharis minima*, therefore, appeared to be storing the assimilates of photosynthesis in the ethanol-soluble fraction presumably as mannitol, whereas *S. vittata* received no ^{14}C-label from *E. maxima*.

5.4 Distribution of Labelled Compounds in the Ethanol-Soluble Fraction

The majority of ^{14}C-labelled assimilates in pooled samples of *L. pallida/C. minima* and *E. maxima/S. vittata* were found in the ethanol-soluble fraction. Table 18 presents the proportion of recovered ^{14}C-label in the ethanol-soluble, alginic acid, laminaran and residue fractions. The recovered radioactivity given in Tables 16 and 17 comes via translocation from the frond, whereas that given in Table 18 is fixed directly by the red algae attached to the brown algal stipe. In both associations more than 80% of the ^{14}C-label was in the ethanol-soluble fraction as was found in the brown algae with no associated red algae (Table 3, Chapter 3). The presence of red algae does not radically alter the distribution of the assimilates of photosynthesis in different fractions of the brown algae. Comparison of Table 18 with Table 3 shows that the percentage recovered ^{14}C-label in the other fractions is also very similar. Alginic acid and laminaran are only present in the brown and not in the red algae.

TABLE 18

The percentage distribution of recovered ^{14}C-label in the ethanol-soluble, alginic acid, laminaran and residue fractions of *L. pallida* with attached *C. minima* and *E. maxima* with attached *S. vittata*. Samples were composed of brown algal stipe with their attached red algae incubated in $NaH^{14}CO_3$ in seawater (1 µCi $m\ell^{-1}$). The residue fraction was that portion remaining after extraction of the first three fractions.

Algal Fraction	*L. pallida/C. minima*	*E. maxima/S. vittata*
* Ethanol-soluble	82,8 (65,4 ± 1,1)	83,9 (66,3 ± 1,0)
* Alginic acid	1,7 (7,5 ± 1,0)	2,0 (8,1 ± 0,9)
* Laminaran	0,9 (5,4 ± 0,6)	1,5 (7,0 ± 0,9)
* Residue	14,6 (22,5 ± 0,9)	12,6 (20,8 ± 1,3)
° Total activity	31,7 ± 1,9	33,4 ± 2,0

* Percentage of total with angular transformation ± SEM in parentheses

° Total activity (DPM x $10^3 g^{-1}$ dry mass ± SEM)

The components of the ethanol-soluble fraction were separated and identified by paper chromatography and gas liquid chromatography. The TMS derivatives of the ethanol-soluble and acid-hydrolysable fractions are given in Figs. 31 and 32 respectively, showing that mannitol is the major component of the ethanol-soluble fraction (46,8 and 47,0mg g^{-1} dry mass) in *L. pallida/C. minima* and *E. maxima/S. vittata* respectively, with the second component being glucose (4,2 and 8,1mg g^{-1} dry mass) in *L. pallida/C. minima* and *E. maxima/S. vittata* respectively (Fig. 31). Comparisons between the brown algae (Fig. 16) and red algae (Fig. 26) show that mannitol and glucose were the main components. The GLC of the acid-hydrolysed components in the *L. pallida/C. minima* association showed that there were three major components: galactose, glucose and mannose (Fig. 32) which had the mol-% of 16,2, 20,8 and 6,3 respectively. The same three components occurred in the *E. maxima/S. vittata* relationship; glucose was present in the largest amount with a mol-% of 19,4, followed by galactose with 15,7 and mannose with 7,2. Comparisons with the brown algae with no attached red algae (Fig. 17, Chapter 3) showed the same three major compounds in the same order of concentration. Mannose was not present in the red algae separated from the brown algae, the two major components being glucose in the greatest concentration followed by galactose. The presence of mannose in the *L. pallida/C. minima* and *E. maxima/S. vittata* associations presumably came from the brown algal component rather than the red algae.

A paper chromatogram of the ethanol-soluble fraction with a glucose marker is shown in Pl. 7A for the stipe of the *L. pallida/C. minima* relationship and Pl. 7B for the stipe of *E. maxima/S. vittata*. The frond of the kelp had been exposed to ^{14}C-label so that its presence in the stipe and red algae was due to translocation. Mannitol was identified in *E. maxima/S. vittata* when an autoradiograph was prepared after six days development; activity was found to be present in mannitol in the ethanol-soluble fraction extracted from the stipe and frond of *Ecklonia maxima* (represented by ES and EF respectively on the autoradiograph, Pl. 8B). The

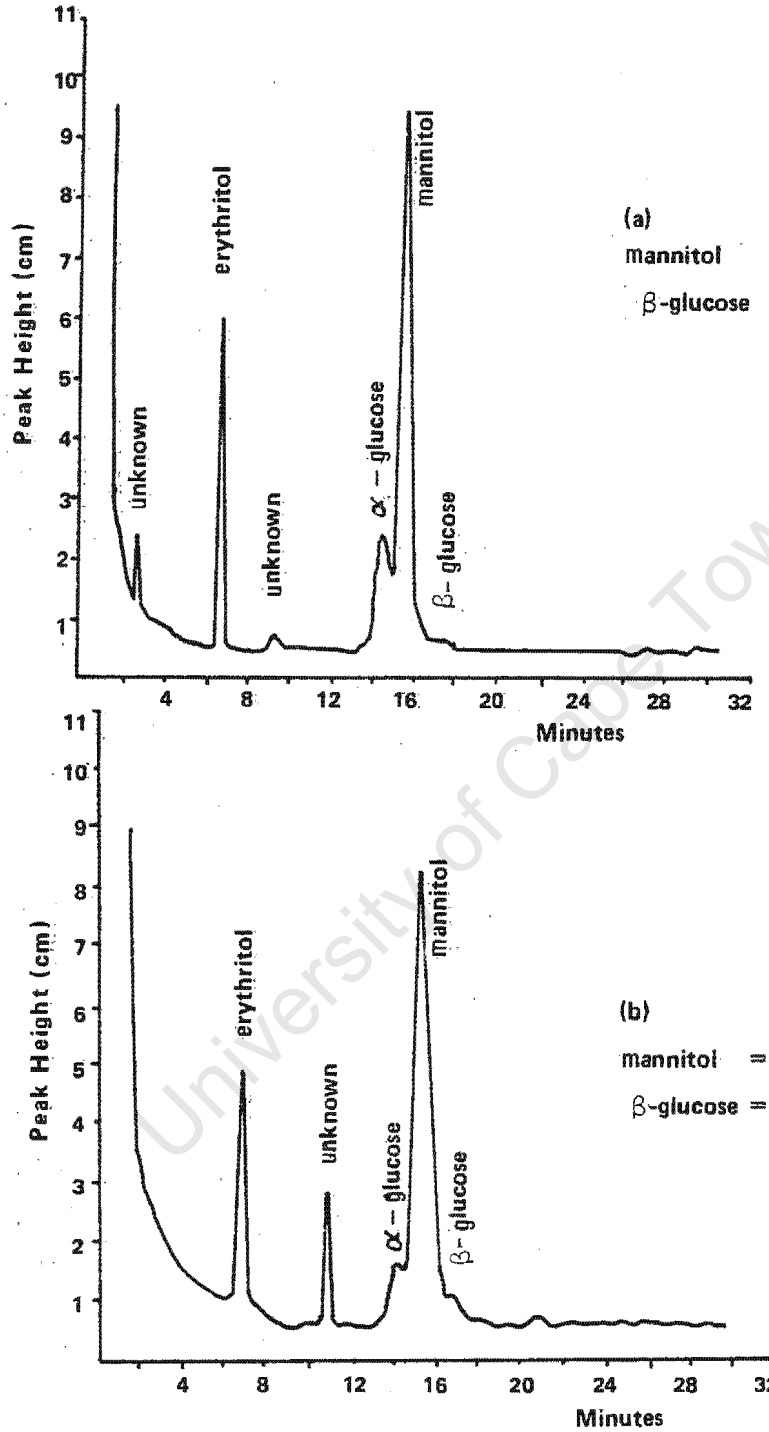


FIG.31 GLC chromatograms of the TMS derivatives of the ethanol-soluble fraction of combined samples of (a) whole plants of *C. minima* and the portions of the stipe of *L. pallida* to which they were attached and (b) whole plants of *S. vittata* and the portions of the stipe of *E. maxima* to which they were attached. Erythritol was used as an internal standard.
* Each is a mean of 3 values. ± SEM

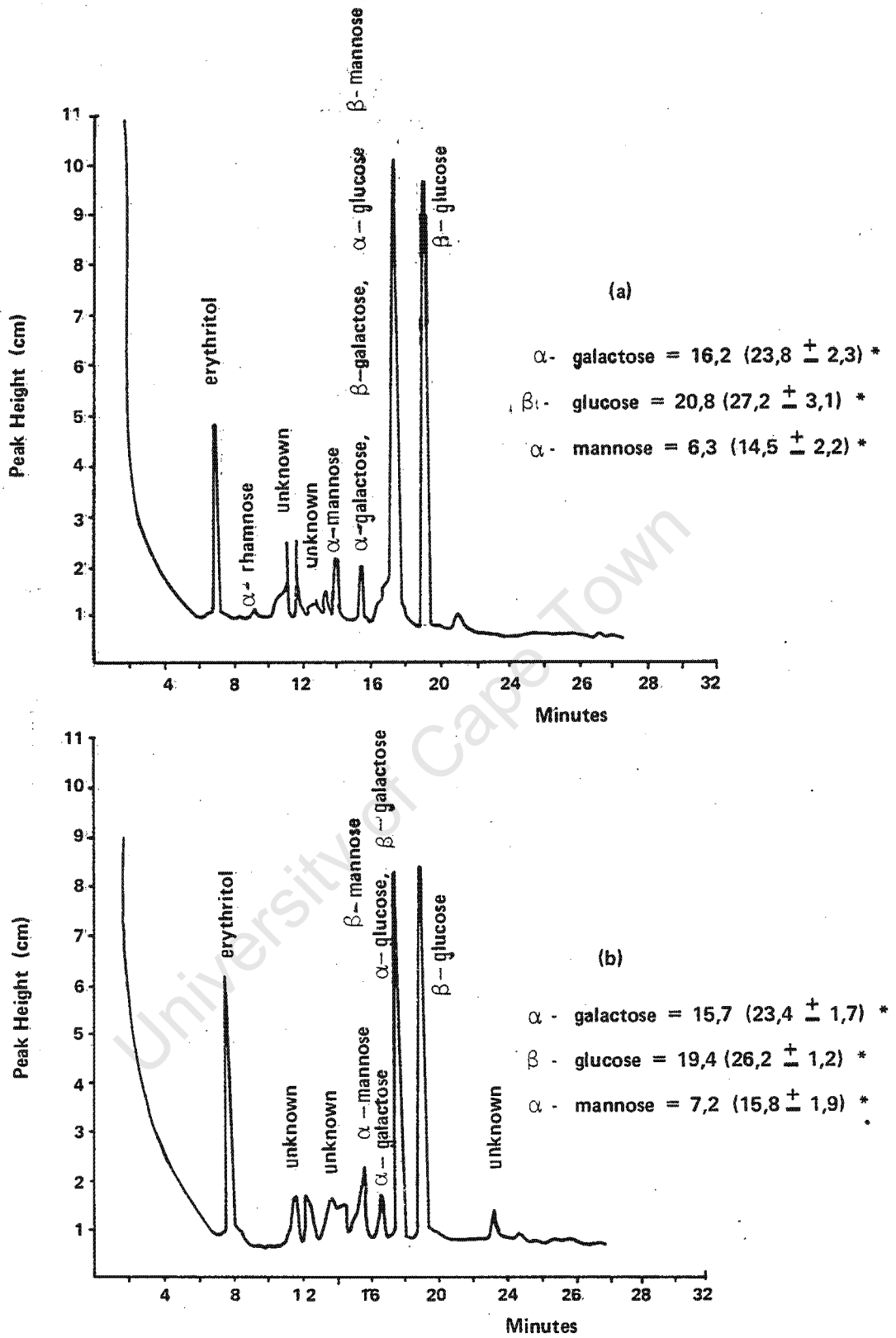


FIG.32 GLC chromatograms of the TMS derivatives of the acid hydrolysed fraction of combined samples of (a) whole plants of *C. minima* and the portions of the stipe of *L. pallida* to which they were attached and (b) whole plants of *S. vittata* and the portions of stipe of *E. maxima* to which they were attached. Erythritol was used as an internal standard. * Expressed as mol - %, angular transformations of percentages are shown in brackets ± SEM. Each is a mean of 3 values.

PLATE 7

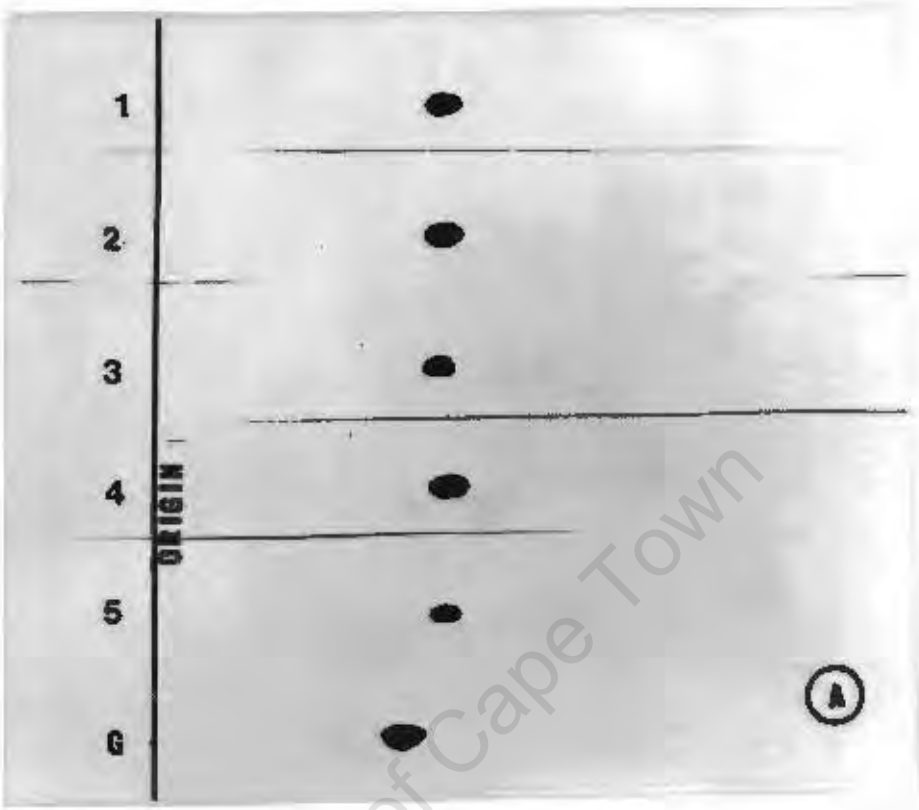
A

A paper chromatogram of an ethanol-soluble extract of *L. pallida* stipe with attached *C. minima* run for thirty eight hours at 20-22°C in ethyl acetate: acetic acid:water (14:3:3) by means of descending chromatography. Carbohydrate spots were detected by the silver nitrate/sodium ethoxide method of Trevelyan *et al.* (1950). The origin is marked, numbers 1-5 are replicates of *L. pallida/C. minima* and G is the standard glucose marker

B

A paper chromatogram of an ethanol-soluble extract of *E. maxima* stipe with attached *S. vittata* run for thirty eight hours at 20-22°C in ethyl acetate: acetic acid:water (14:3:3) by means of descending chromatography. Carbohydrate spots were detected by the silver nitrate/sodium ethoxide method of Trevelyan *et al.* (1950). The origin is marked, numbers 1-5 are replicates of *E. maxima/S. vittata* and G is the standard glucose marker

PLATE 7



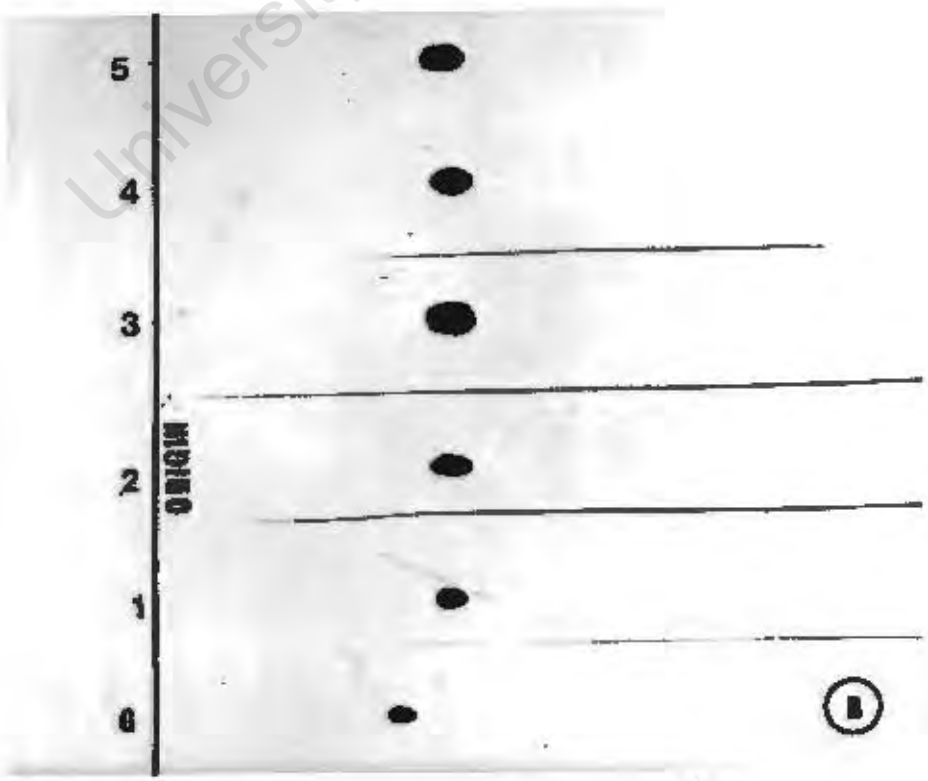


PLATE 8

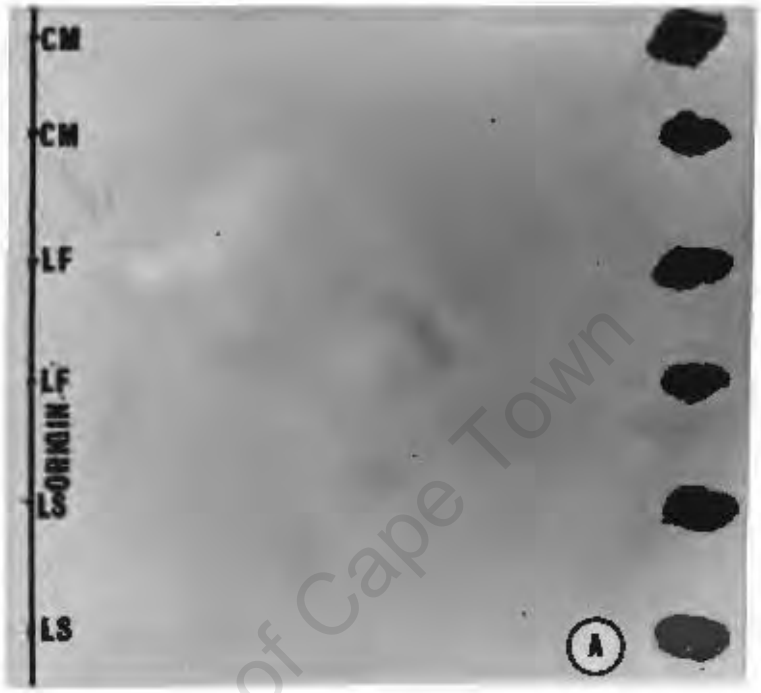
A

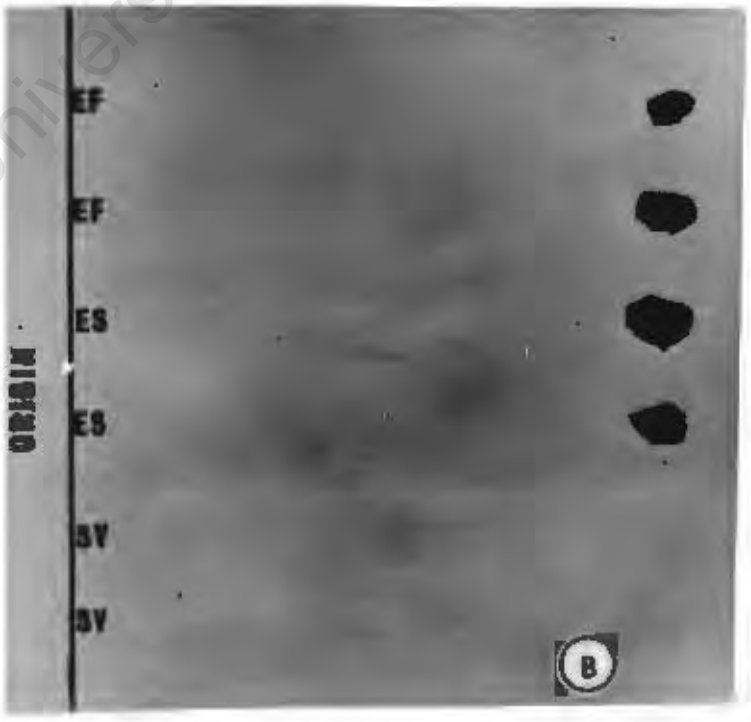
An autoradiograph on Kodak X-ray film prepared from an ethanol-soluble fraction of *L. pallida* with attached *C. minima*. The autoradiograph was left for six days to develop. The different fractions are represented by the frond of *L. pallida* (L.F.), the stipe of *L. pallida* (L.S.) and the attached red alga (C.M.)

B

An autoradiograph on Kodak X-ray film prepared from an ethanol-soluble fraction of *E. maxima* with attached *S. vittata*. The autoradiograph was left for six days to develop. The different fractions are represented by the frond of *E. maxima* (E.F.), the stipe of *E. maxima* (E.S.) and the red alga (S.V.)

PLATE 8





ethanol-soluble extract of *S. vittata* (designated SV in Pl. 8B) showed no activity even when left to develop for twenty one days. A typical autoradiograph of *L. pallida/C. minima* is shown in Pl. 8A where the radioactive component which could be identified was mannitol after incubation in $NaH^{14}CO_3$ seawater and photosynthesis by the frond.

5.5 Analysis of "Free" Amino Compounds

The "free" amino compounds found in the stipe of *L. pallida* with attached *C. minima* and the stipe of *E. maxima* with attached *S. vittata* are given in Table 19 and the distribution of ^{14}C in various amino acids in Table 20. In both associations alanine is the major "free" amino acid which corresponds to the results for the brown algae with no attached red algae (Table 4, Chapter 3) and for red algae alone (Table 12, Chapter 4) the major "free" amino acid being alanine, followed by glutamic acid and glutamine. In experiments where the frond of the brown algae was exposed to ^{14}C-label in seawater the "free" amino acids in the frond, stipe and red algae were analysed on the amino acid analyser to ascertain which contained ^{14}C-label. In all cases alanine carried the majority of the ^{14}C-label followed by aspartic acid, except in *S. vittata* where no ^{14}C-label was present (Table 20).

5.6 Anatomy of the Penetration of the Red Algae into the Brown Algae

When a section of brown algal stipe, which had red algae attached to it, was cut transversely a total difference in the degree of penetration was noticed between *C. minima* and *S. vittata*. In *C. minima* there was a deep penetration of the red alga into the stipe of *L. pallida* (Pl. 9A). This peg shaped penetration entered the stipe of the brown alga to a distance equivalent to a quarter of its diameter but appeared to be intercellular under the light microscope. The red alga formed a flat disc-like base, in the *E. maxima/ S. vittata* relationship, which was attached to the surface of the brown algae but did not penetrate at all the cortical cells of the stipe (Pl. 9B).

TABLE 19

Concentration of "free" amino acids in μmoles g^{-1} fresh mass in the *L. pallida/C. minima* and *E. maxima/S. vittata* associations. Samples were of brown algal stipe and attached red algae.

Amino Acids	*L. pallida* Red algae present	*E. maxima* Red algae present
Aspartic acid	0,357	0,291
Threonine	0,074	0,067
Serine	0,179	0,193
Asparagine	0,102	0,083
Glutamic acid	0,842	0,654
Glutamine	0,537	0,485
Glycine	0,134	0,274
Alanine	1,907	1,841
Valine	0,034	0,034
Cystine	0,067	0,019
Methionine	0,051	0,096
Isoleucine	0,013	0,020
Leucine	0,011	0,027
Tyrosine	0,023	0,027
Phenylaline	0,090	0,036
Lysine	0,011	0,146
Histidine	0,430	0,302

TABLE 20

The distribution of radioactivity in various amino acids in *L. pallida/C. minima* and *E. maxima/S. vittata* associations. The fronds of each plant were incubated in $NaH^{14}CO_3$ for six hours and results are means of three separate experiments expressed as DPM g^{-1} fresh mass (g^{-1} fm). Samples were of brown algal stipe and red algae and obtained on a Beckman 120C Amino Acid Analyser.

Amino Acids labelled with ^{14}C-label	*L. pallida/C. minima*			*E. maxima/S. vittata*		
	Frond DPM g^{-1} fm	Stipe DPM g^{-1} fm	*C. minima* DPM g^{-1} fm	Frond DPM g^{-1} fm	Stipe DPM g^{-1} fm	*S. vittata* DPM g^{-1} fm
Alanine	5853	8170	3400	14067	1513	NA
Aspartic acid	1023	1644	997	881	593	NA
Asparagine	547	747	367	531	39	NA
Glycine	138	366	190	766	127	NA
Threonine	78	419	30	915	191	NA
Glutamine	248	324	138	280	30	NA
Glutamic acid	310	219	126	237	25	NA

NA = No activity present

opposite page 115

PLATE 9

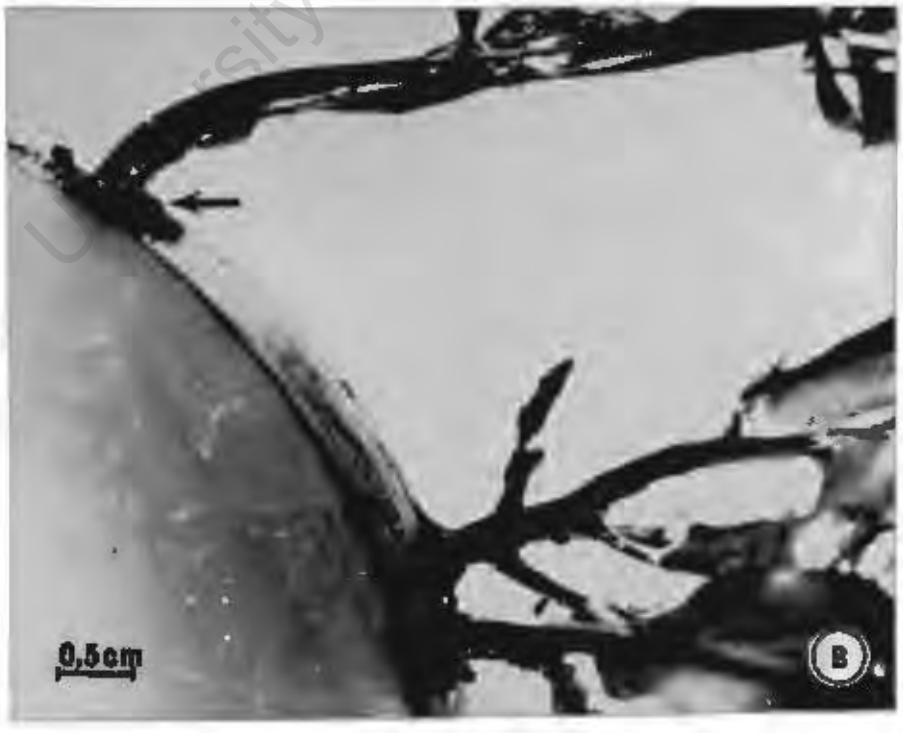
A

A section through the stipe of *L. pallida* showing the penetration of *C. minima* (arrowed) into the brown alga

B

A section through the stipe of *E. maxima* showing that *S. vittata* is attached to the brown alga by means of a flat cushion-like structure (arrowed) but does not penetrate into the stipe

PLATE 9



5.7 Discussion

The movement of the ^{14}C-labelled assimilates of photosynthesis from frond to stipe and then to the red algae, revealed a major difference between the *L. pallida/C. minima* and *E. maxima/S. vittata* associations. In the former 8,6% of the exported activity occurred in *C. minima*, whereas in the latter it was only 0,3% (Table 13). These results suggest an active movement from *L. pallida* to *C. minima*. This is in contrast to the results of translocation in the kelp with no attached red algae, where *L. pallida* exhibited a velocity of 50-100mm h^{-1} and *E. maxima* 240-300mm h^{-1} (Fig. 14 and Fig. 15 respectively). Schmitz and Lobban (1976) state that 50mm h^{-1} was much faster than diffusion. *Ecklonia maxima* possessed a more complex arrangement of hyphal threads (Pl. 3A and 3B) than *L. pallida* (Pl. 2A and 2B) and was the probable reason for the faster translocation velocity in *E. maxima* although both brown algae actively translocated.

The presence of red algae upon the stipe of the brown algae does not appear to drastically affect the distribution of the assimilates of photosynthesis. A similar pattern of distribution in the various regions of the plant is shown in Table 1 (brown algae with no attached red algae) and Table 13 (brown algae with attached red algae). Comparison of Table 3 with Table 15 indicates that the presence of the red algae does not affect the distribution of ^{14}C-label within different fractions of the brown algae with the majority occurring in the ethanol-soluble fraction. There was no transfer to *S. vittata* but in the case of *C. minima* transfer occurred from the stipe of *L. pallida* and the more *C. minima* plants present the greater the transfer (Table 14). The movement of the ^{14}C-labelled assimilates of photosynthesis from *L. pallida* to *C. minima* has also been demonstrated in several other associations. Harlin (1973b) showed transfer in both directions of ^{14}C and ^{32}P between the red alga *Smithora naiadum* and the sea grasses to which it was attached. In *Choreocolax* attached to *P. lanosa*, there was a consistent increase in total radioactivity in attached plants of *Choreocolax*. This increase occurred despite the absence of any external label and after an initial period of

photosynthesis by the host (Callow *et al.*, 1979). The *Choreocolax* was able to fix CO_2 photosynthetically, an ability which appeared to be partially repressed when attached to the host (Callow *et al.*, 1979). Evans *et al.*, (1973) proposed that carbon was translocated from *Gracillaria* to *Holmsella* which was converted in the parasite to mannitol and starch. *Odonthalia* tissue, infected with *Harveyella*, showed a greater incorporation of $^{14}CO_2$ than uninfected tissue, consistent with an increased rate of photosynthesis (Goff, 1976). Harlin (1973a) has demonstrated that *Smithora naiadum* will grow on an artificial substratum, although it has only ever been observed in the field attached to sea grasses but not on other plants, shellfish or rocks.

The ^{14}C-labelled compounds in the *L. pallida/C. minima* and *E. maxima/S. vittata* associations were mannitol and certain amino acids after photosynthetic ^{14}C-assimilation. Mannitol was the major compound translocated in the kelp (Chapter 3) and was found to be present in both red algae (Chapter 4). Mannitol is widespread in the Phaeophyceae (Craigie, 1974; Lewis and Smith, 1967a) and reported in several species of Rhodophyceae (Craigie, 1974; Lewis and Smith, 1967a; Majak *et al.*, 1966). Kremer (1976a) stated that many reports of mannitol in red algae were due to incorrect identification, although the presence of ^{14}C-labelled mannitol and floridoside was convincingly identified by modern methods in *Holmsella* (Evans *et al.*, 1973). Kremer (1976a) considered that this red alga living parasitically may not exhibit the normal metabolic characteristics. Callow *et al.* (1979) found that in the association of *Choreocolax* on *P. lanosa* the products of photosynthesis may include mannitol and an unidentified complex carbohydrate. The major product of photosynthesis in the host *Polysiphonia* was sodium mannoglycerate, a compound restricted to the Ceramiales (Evans *et al.*, 1978). Sodium mannoglycerate in *Choreocolax* was due to contamination from the displaced *Polysiphonia* cells (Evans *et al.*, 1978) and there was an accumulation of ^{14}C-label in insoluble materials in both host and parasite. In *C. minima* and *S. vittata* there was only evidence of ^{14}C-label in mannitol and certain amino acids. It was impossible to identify

sodium mannoglycerate or floridoside as their high molecular weights prevented separation by GLC or paper chromatography. It was considered that this was not a serious omission as previous workers found that where mannitol accumulated in any quantities in red algae, for example the Ceramiales, floridoside was only present in very small amounts (Craigie, 1974) and where floridoside was the major storage product mannitol was not present. Floridoside, the commonest carbohydrate in red algae, has not been identified in brown algae (Craigie, 1974; Percival and McDowell, 1967). Exogenously supplied mannitol was assimilated by both *C. minima* and *S. vittata* (see Chapter 4) but the majority of studies to date on the incorporation of exogenously supplied mannitol, have been limited to brown algae (Bidwell, 1967; Bidwell and Ghosh, 1962; Yamaguchi *et al.*, 1966). Exogenously supplied mannitol was poorly utilized by *Fucus vesiculosus* (Bidwell and Ghosh, 1962) whereas endogenously supplied mannitol was readily metabolised and interconvertible with laminaran in both *Fucus* and *Eisena* (Bidwell, 1967; Yamaguchi *et al.*, 1966).

The percentage of ^{14}C-label supplied in the form of ^{14}C-mannitol in the various fractions of *C. minima* remained relatively constant over the time course of twenty four hours (Table 10, Chapter 4). In *S. vittata*, however, there was a decrease in the percentage of ^{14}C-label in the ethanol-soluble fraction over the same time period (Table 10, Chapter 4). This suggested that the ^{14}C-label in *S. vittata* was being metabolised into the starch-containing fraction and stored, whereas in *C. minima* it was stored in the ethanol-soluble fraction presumably in the form of mannitol. The percentage label in the acid-hydrolysed fraction remained approximately the same in both algae and this would be expected as this fraction arises from breakdown of stable components, e.g., the cell wall. In the time course studies using whole sporophylls of the brown algae, and analysing the percentage ^{14}C-label in the various fractions of red algae and brown algal stipe (Table 16) *Carpoblepharis minima* appeared to store its assimilates of photosynthesis in the ethanol-soluble fraction, probably as the acyclic

polyol mannitol. The ^{14}C-label in *S. vittata* was converted into the starch-containing fraction and more detailed analysis would be required to elucidate the pathway and final product of the ^{14}C-label incorporation. When pooled samples of brown algal stipe/red algae (Table 16) and isolated red algae (Table 17) were analysed for the percentage recovered ^{14}C-activity in the starch-containing fraction, it was not as high as that shown in Table 18. It must be remembered, however, that in the former experiments only the frond was exposed to ^{14}C-label and the recovered ^{14}C-label was present by translocation, whereas in the latter experiments the red algae were in direct contact with the ^{14}C-label in seawater. In the translocation experiments *C. minima* obtained ^{14}C-labelled assimilates of photosynthesis from *L. pallida* (Table 17) but *S. vittata* obtained no ^{14}C-label from *E. maxima* (Table 17). Both red algae were capable of fixing ^{14}C-labelled assimilates by photosynthesis from exogenously supplied mannitol (Table 10) and $NaH^{14}CO_3$ in seawater (Table 18).

Comparison of the results of paper chromatography, GLC and amino acid analysis of brown algae with no attached red algae (Chapter 3) isolated red algae (Chapter 4) and the brown/red algal association (this chapter) showed that there were no significant differences between them. Mannitol was always the major component of the ethanol-soluble fraction. In the GLC experiments, mannitol gave results of 53 and 38,5mg g^{-1} dry mass in the stipe of *L. pallida* and *E. maxima* with no attached red algae, respectively (Fig. 16, Chapter 3) 44 and 41,5mg g^{-1} dry mass in *C. minima* and *S. vittata*, respectively (Fig. 28, Chapter 4) 47mg g^{-1} dry mass in the stipe of *L. pallida/C. minima* and 47,7mg g^{-1} dry mass in the stipe of *E. maxima/S. vittata* (Fig. 31). Newell *et al.* (1980) in an analysis of the mucilage of *L. pallida* and *E. maxima*, found that mannitol comprised 76,8% of the total in the former and 77,5% of the total in the latter. The acid hydrolysed fraction showed the presence of glucose, galactose and mannose in both kelp with no red algal association (Fig. 17) and in the kelp with attached red algae (Fig. 32). In the GLC of the red algae with no kelp present, only glucose

and galactose were identified (Fig. 29); presumably the presence of mannose in the samples of kelp and red algae combined came from the brown algal component and not from the red algae. Newell *et al.* (1980) reported the presence of α- and β-galactose, α- and β-fucose and trace amounts of mannose in the acid-hydrolysed fraction of the mucilage of *L. pallida* and *E. maxima*. Percival (1978) has reported the presence of both mannose and galactose in some species of brown algae, while the reserve polysaccharide of brown algae is the 1→3-linked β-D-glucan, laminaran, samples of which from different orders have been submitted to detailed chemical investigation with the object of establishing minor structural variations (Percival, 1968). The major polysaccharides in red algae are the galactans which all contain galactose. Rees (1969) showed that they all appeared to consist of chains of alternating units of 1,3-linked β-galactose and 1,4-linked α-galactose. It was not established, or even considered likely, that the components of the acid-hydrolysed fraction were translocated. There were a greater number of unknown compounds identified by GLC in the ethanol-soluble and acid-hydrolysed fractions of both red algae when isolated (Fig. 28 and Fig. 29, Chapter 4) than in either brown algae alone (Fig. 16 and Fig. 17, Chapter 3) and pooled samples of the brown/red algal association (Fig. 31 and Fig. 32). Several of the unknown compounds found in the samples of the association had the same retention times as those in the red algae and therefore arose from this partner of the association. These compounds could not be assimilates of photosynthesis transferred from the brown algae to the red algae with which it grows in association.

The three "free" amino acids found in order of concentration were alanine, glutamine and histidine in *C. minima* and glutamine, glutamic acid and glycine in *S. vittata* (Table 12, Chapter 4). In the kelp with no attached red algae they were alanine, glutamic acid and histidine in *L. pallida* and alanine, glutamic acid and glutamine in *E. maxima* (Table 4, Chapter 3). Alanine was the major "free" amino acid in all cases except *S. vittata* and when labelled with ^{14}C after photosynthesis in $NaH^{14}CO_3$, alanine carried the greatest

^{14}C-label followed by aspartic acid and asparagine (Table 20). There was no ^{14}C-label in *S. vittata* as it was not transferred from *E. maxima* and it was, therefore, impossible to know which, if any, amino acid would be labelled after photosynthesis. It was not established whether these ^{14}C-labelled amino acids were translocated or synthesised *in situ*. Parker (1966) in an analysis of the exudate of *Macrocystis* gave the compounds which were translocated as mannitol and the amino acids alanine, aspartic acid and glutamic acid. Schmitz *et al.* (1972) also reported translocated compounds to be composed of mannitol and a variety of amino acids including alanine, glycine, serine and glutamic acid in *L. saccharina* and *L. hyperborea*. Parker (1966) found only mannitol carried the ^{14}C-label after photosynthesis. The presence of alanine as the major "free" amino compound suggested that it was translocated, but this could not be conclusively established as exudate of *L. pallida* and *E. maxima* could not be obtained. Hellebust and Haug (1972) indicated that the amino acids were the most likely sources of carbon for alginic acid synthesis and respiration in the dark when mannitol is not readily available.

The results given in this chapter have shown that *S. vittata* receives minimal amounts of the assimilates of photosynthesis from the stipe of *E. maxima*; 0,3% of the recovered ^{14}C-label after translocation from the frond to the stipe. The ^{14}C-label recovered was so little due to passive diffusion, whereas *C. minima* received a far greater percentage of the recovered radioactivity (8,6%). The greater the number of *C. minima* plants present on one *L. pallida* plant the more transfer occurred (Table 14) as the red algae act as sinks for the assimilates of photosynthesis from the brown algae. The degree of colonization appeared to be a seasonal factor probably related to the growth of the kelp. Another factor which probably contributes to the degree of colonization is the severe winter storms in the South-western Cape waters which cause extensive damage to the kelp and tear off the red algae. Both red algae, however, are able to take up exogenously supplied mannitol (Table 10, Chapter 4) and photosynthetically fix ^{14}C supplied as $NaH^{14}CO_3$ (Fig. 24

and Fig. 25, Chapter 4) and have the normal photosynthetic pigments of red algae (Fig. 26 and Fig. 27, Chapter 4). All of this would indicate that *S. vittata* and *C. minima* are able to lead an autotrophic existence, but *C. minima* actively obtained assimilates of photosynthesis from *L. pallida*.

CHAPTER 6

AN EVALUATION OF THE *LAMINARIA PALLIDA/CARPOBLEPHARIS MINIMA* AND *ECKLONIA MAXIMA/SUHRIA VITTATA* ASSOCIATIONS

This study has investigated the association between *L. pallida* and the red alga, *C. minima*, which grows attached to it, and *E. maxima* and its attached red alga *S. vittata*. The pathology of marine algae has been considered a neglected field (Andrews, 1976) while the identification of the red algal partner as either an epiphyte or parasite was based on anatomical and morphological rather than physiological studies (Evans *et al.* 1978). Earlier studies used such criteria as penetration of host tissue, reduction of thallus, loss of colour and host specificity (Feldmann and Feldmann, 1958; Setchell, 1918). In many taxonomic studies, any alga which grows attached to another alga is termed an epiphyte or a parasite with no experimentation having been made into the nutritional status of the algae. (A review of red algal parasites is given by Evans *et al.*, 1978). More recent studies have used physiological and biochemical techniques in a re-examination of certain alloparasites (Callow *et al.*, 1979; Evans *et al.*, 1973, 1978; Goff, 1976; Goff and Cole, 1976; Kugrens and West, 1973) and adelphoparasites (Court, 1977; Goff, 1977; Kugrens, 1970; Kugrens and West, 1972a, 1972b, 1973). Some of these modern studies have demonstrated that the algae were correctly termed parasites; for example, Goff (1976) showed that ^{14}C-assimilated by the host was transferred primarily from medullary cells to the adjacent rhizoids of the alloparasite *Harveyella mirabilis*. Various membrane systems of the rhizoids, including endoplasmic reticulum, pinocytotic vesicles and dictyosomes, may be involved in the uptake and distribution of nutrients.

The order Ceramiales, which includes *C. minima*, contains a large number of parasitic red algae (reviewed by Evans *et al.*, 1978). These are not well represented in the Gelidiaceae which includes *S. vittata*. Parasitic members of the Ceramiales have been described by Batters (1892)

Dawson (1944) and Martin and Pocock (1953). In the alloparasite *Holmsella* the soluble ^{14}C-labelled carbohydrates were floridoside and mannitol, whereas in its host *Gracillaria* the ^{14}C-labelled, soluble carbohydrate was floridoside (Evans *et al.*, 1978). *Holmsella pachyderma* forms smooth, white cushions totally lacking in pigment on *Gracillaria*. A time study (Evans *et al.*, 1973) showed an increase in ^{14}C-label recovered from the parasite with time implying a translocation of fixed carbon from host to parasite. Evans *et al.* (1973) proposed that carbon was translocated from host to parasite in the form of floridoside, which was then converted in the parasite to mannitol and starch. In another alloparasite *Choreocolax* growing upon *Polysiphonia lanosa*, the degree of contact with the host plant is greater than in the *Holmsella/Gracillaria* association. *Polysiphonia* cells are displaced deeply into the *Choreocolax* thallus and secondary pit connections are present. Host cells undergo hypertrophy, hyperplasia and cell destruction although this is localised and does not appear to affect the host. In the case of *S. vittata* there was no penetration into the brown algal stipe and instead a flat cushion-like structure was formed on the surface of the stipe (Pl. 9B). *Carpoblepharis minima* did penetrate the stipe of *L. pallida* (Pl. 9A). Harlin and Craigie (1975) and Turner and Evans (1977) established that there was no correlation between either the degree of attachment or the amount of assimilates of photosynthesis transferred between host and parasite. Harlin (1973b) demonstrated transfer in both directions of ^{14}C and ^{32}P between a red alga *Smithonia naiadum* and the sea grass to which it was attached, despite its limited attachment in the form of a flat basal cushion. In contrast, *Polysiphonia lanosa*, which is firmly attached by rhizoids which penetrate the host *Ascophyllum nodosum* (Rawlence, 1972) no large scale directional translocation has yet been demonstrated (Harlin and Craigie, 1975; Turner and Evans, 1977). The major product of photosynthesis in *Polysiphonia* is another glycoside, sodium mannoglycerate, and in *Choreocolax* most of the radioactivity in the soluble component was in sodium mannoglycerate and an unidentified complex sugar (Evans

et al., 1978). Callow *et al.* (1979) discovered the ability of *Choreocolax* to fix CO_2 photosynthetically, an ability which appears to be partially repressed in the attached state since CO_2 fixation progressively increases with time if *Choreocolax* plants are detached from the host. The products of *Choreocolax* photosynthesis may include mannitol and an unidentified complex carbohydrate (Callow *et al*, 1979). In the *L. pallida/C. minima* association, mannitol and certain amino acids were the only ^{14}C-labelled compounds present in the brown and red algae. There was a transfer of the ^{14}C-labelled assimilates of photosynthesis from *L. pallida* to *C. minima* in great enough quantities to be due to active movement. In the *E. maxima/S. vittata* association there was slight transfer of the ^{14}C-labelled assimilates of photosynthesis from brown to red algae, but the quantities were small enough to be due to passive diffusion. The only compounds identified by paper chromatography, GLC and autoradiography to carry the ^{14}C-label were mannitol and certain amino acids, the major ones being alanine, aspartic acid and asparagine. Mannitol was found to be a major component in both brown and red algae with alanine as the major "free" amino acid in all cases. Although mannitol is a widespread component in the Phaeophyceae and has been reported in the Rhodophyceae, Kremer (1976b) stated that its presence may have been due to incorrect identification. He reported that the presence of mannitol in the parasite *Holmsella* had been conclusively proved by Evans *et al.* (1973) but stated that as a parasite this may not be the normal metabolic state. Plants which live in a close association with another plant may well adapt and evolve to utilize the nutrients of the host and thus no longer show the representative carbohydrates of the group in which they are taxonomically classified.

There is an essential difference in the behaviour of the two red algae *C. minima* and *S. vittata* chosen for this study (see Chapter 1). This was confirmed by the ^{14}C-labelling experiments as mannitol was transferred in the case of the *L. pallida/C. minima* relationship, but not in the case of the *E. maxima/S. vittata* association. Both

red algae possessed mannitol as a major component and incorporated exogenously supplied mannitol. In addition, as both red algae contained all the photosynthetic pigments necessary for an autotrophic existence, neither was totally parasitic. The interpretation of ^{14}C-photosynthesis and translocation experiments is difficult if there is some degree of independent fixation by the parasite. In addition, the metabolism of the host in the region of the parasite may be affected, especially in those situations where hypertrophy or hyperplasia create additional metabolic sinks (Evans *et al.*, 1978). The presence of a greater number of *C. minima* plants does cause a greater percentage of the recovered ^{14}C-label to be translocated from the frond to the stipe and then to the red algae (Table 14, Chapter 5). There was active transfer of recovered ^{14}C-label from the frond to the stipe of *E. maxima* but the number of *S. vittata* plants attached to the stipe did not affect this pattern of transfer and very little of the ^{14}C-label was recovered in the red algae, being low enough (less than 0,5%) to be due to diffusion (Table 15, Chapter 5).

The term "epiphyte" has been loosely applied to describe the relationship of the red algae to the kelp, but it is evident from this study that this should be reviewed as it is important, when describing any relationship, to define the terminology. Evans *et al.* (1978) stated that the term "epiphyte" should be restricted to algae which were totally independent of their host for nutrient requirements. The host alga would then be termed a "basiphyte" (Linskens, 1963). "Epiphytes", while using other plants for mechanical support, do contribute to primary productivity (Ballantine, 1979; Brock, 1970) are sources of food for grazers (Berg, 1975; Reyes-Vasquez, 1970) and comprise a major portion of the species within the ecosystem (Ballantine and Humm, 1975). A plant parasite, on the other hand, has been defined by the Federation of British Plant Pathologists (1973) as "an organism or virus existing in intimate association with a living organism from which it derives an essential part of the material for its existence whilst conferring no benefit in return". This definition differs from that of Goff (1976) in that parasitism

is clearly separated from pathogenesis, i.e., the ability of the parasite to cause disease in the host. Lewis (1974) has proposed a classification of nutritional categories which would be applicable to all chemoheterotrophic organisms, plant, animal and microbe. This, however, assumes that organisms which derive organic compounds directly from another organism do so by virtue of permanent, intimate contact and is known as synbiotrophy. The presence or absence of secondary pit connections must be considered doubtful in assessing a red algal host/parasite relationship as red algal pit connections are not thought to function in the movement of substances between cells (Evans *et al.*, 1978). These workers also state that an association would still constitute parasitism if there was an obligate dependence on the part of the one organism for minor metabolites, growth factors or vitamins supplied by the other partner.

Applying these definitions of epiphytes and parasites to the *E. maxima/S. vittata* and *L. pallida/C. minima* associations, the following conclusion may be obtained. The red alga derived a very small proportion of the recovered ^{14}C-label fix by photosynthesis of the frond of *E. maxima*. These assimilates of photosynthesis were translocated to its stipe to which the red algae grew attached. Any ^{14}C-label recovered in *S. vittata* was due to passive diffusion across a concentration gradient or leakage across cells and was not due to active transport. *Suhria vittata* was, therefore, considered to be an epiphyte on *E. maxima*, using it as a substratum and capable of an autotrophic existence. It was not dependent on the brown algae for any nutrients, supported by its ability to grow on rocks and shellfish.

Carpoblepharis minima did receive ^{14}C-labelled assimilates of photosynthesis from *L. pallida* in too large a quantity to be due to a passive process. The number of red algal plants affected the pattern of distribution of these assimilates indicating the close inter-relationship between these two algae. Using the definition of Evans *et al.* (1978) formulated after extensive studies on red algal parasites, by themselves and all previous workers in the field, *C. minima* would be correctly termed a parasite being

nutritionally dependent on *L. pallida*, supported by the fact that it has never been observed growing on any other substratum than the brown algae, *L. pallida*. Despite being attached to a specific host, and parasitic on it, *C. minima* was capable of undergoing autotrophic nutrition.

REFERENCES

ANDREWS, J.H. (1976). The pathology of marine algae. *Biol. Rev.*, **51**, 211-253.

APARICHIO, P.J.. CÁRDENAS, J., ZUMFT, W.G., VEGA, J.M., HERRERA, J., PANEQUE, A. and LOSADA, M. (1971). Molybdenum and iron as constituents of the enzymes of the nitrate reducing system from *Chlorella*. *Phytochem.*, **10**, 1487-1495.

ATKIN, G.E. and FERDINAND, W. (1970). Accelerated amino acid analysis. Studies on the use of lithium citrate buffers and the effect of n-propanol in the analysis of physiological fluids and protein hydrolyzates. *Anal. Biochem.*, **38**, 313-330.

BALLANTINE. D.L. (1979). The distribution of algal epiphytes on macrophyte hosts offshore from La Parguera, Puerto Rico. *Bot. mar.*, **22**, 107-111.

BALLANTINE, D.L. and HUMM, H.J. (1975). Benthic algae of the Anclote Estuary. I. Epiphytes of seagrass leaves. *Fla. Sci.*, **38**, 150-162.

BARLOW, R.G. (1980). The biochemical composition of phytoplankton in an upwelling region off South Africa. *J. exp. mar. Biol. Ecol.*, **45**, 83-93

BARLOW, R.G. (1981). Phytoplankton in a nearshore region of the southern Benguela current. Ph.D. thesis, University of Cape Town.

BATTERS, E.A.L. (1892). *Gonimophyllum buffhami*: a new marine alga. *J. Bot. (Lond.)*, **30**, 65-67.

BEEVERS, L. and HAGEMAN, R.H. (1969). Nitrate reduction in higher plants. *A. Rev. Pl. Physiol.*, **20**, 495-522.

BERG, J. (1975). Behaviour and ecology of conch (Superfamily Strombacea) on a deep subtidal algal plain. *Bull. mar. Sci.*, **2**, 307-317.

BIDWELL, R.G.S. (1967). Photosynthesis and metabolism in marine algae. VII. Products of photosynthesis in fronds of *Fucus vesiculosus* and their use in respiration. *Can. J. Bot.*, **45**, 1557-1565.

BIDWELL, R.G.S. and GHOSH, N.R. (1962). Photosynthesis and metabolism in marine algae. IV. The fate of ^{14}C-mannitol in *Fucus vesiculosus*. *Can J. Bot.*, **40**, 803-807.

BIDWELL, R.G.S. and GHOSH, N.R. (1963). Photosynthesis and metabolism in marine algae. V. Respiration and metabolism of ^{14}C-labelled glucose and organic acids supplied to *Fucus vesiculosus*. *Can. J. Bot.*, **41**, 155-163.

BIDWELL, R.G.S., PERCIVAL, E.G.V. and SMESTAD, B. (1972). Photosynthesis and metabolism in marine algae. VIII. Incorporation of $^{14}CO_2$ into the polysaccharide metabolised by *Fucus vesiculosus* during pulse labelling experiments. *Can. J. Bot.*, **50**, 191-197.

* BLACK, W.A.P., CORNHILL, W.J., DEWAR, E.T. and WOODWARD, F.N. (1951). Manufacture of algal chemicals. III. Laboratory scale isolation of laminaran from brown marine algae. *J. appl. Chem.*, **1**, 505-517.

BROCK, T.D. (1970). Photosynthesis by algal epiphytes of *Utricularia* in Everglades National Park. *Bull. mar. Sci.*, **20**, 952-956.

BRYLINSKY, M. (1977). Release of dissolved organic matter by some marine macrophytes. *Mar. Biol.*, **39**, 213-220.

BROWN, P.C. (1980). Phytoplankton production studies in the coastal waters off the Cape peninsula, South Africa. MSc. thesis, University of Cape Town.

BUGGELN, R.G. (1976). The rate of translocation in *Alaria esculenta* (Laminariales, Phaeophyceae). *J. Phycol.*, **12**, 439-442.

BUGGELN, R.G. (1977). Physiological investigations on *Alaria esculenta* (Laminariales, Phaeophyceae). II. Role of translocation in blade growth. *J. Phycol.*, **13**, 212-218.

BUGGELN, R.G. (1981). A general method for determining translocation velocities in Laminariales. *Can. J. Bot.*, **59**, 132-136.

CALLOW, J.A., CALLOW, M.E. and EVANS, L.V. (1979). Nutritional studies on the parasitic red alga *Choreocolax polysiphoniae*. *New Phytol.*, **83**, 451-462.

CAMERON, M.C., ROSS, A.G. and PERCIVAL, E.G.V. (1948). Method for routine estimation of mannitol, alginic acid and combined fucose in seaweeds. *J. Soc. chem. Ind. (Lond.)*, **67**, 161-165.

CANNY, M.J. (1960). The rate of translocation. *Biol. Rev. Cambridge Philos. Soc.*, **35**, 507-532.

CANNY, M.J. (1973) *Phloem Translocation*. pp 144-148. Cambridge University Press, Cambridge.

CÁRDENAS, J., RIVAS, J., PANEQUE, A. and LOSADA, M. (1971). Molybdenum and the nitrate reducing system from *Chlorella*. *Arch. Mikrobiol.*, **79**, 367-376.

COASSINI LOKAR, L.C. and BARADEL, P. (1967). Sugli oligosaccaridi e sui polisaccaridi delle alghe del'alto Adriatico. III. Determinazione del contenuto di mannitolo nel *Fucus virsoides*. *Univ. Stude trieste, Fac. Econ. Commer. 1st Merceol.*, **32**, 5-21.

COURT, G.J. (1977). The symbiotic association of the red algae *Janczewskia gardneri* and *Laurencia spectabilis* (Ceramiales, Rhodophyceae) *J. Phycol.*, **13**(suppl.), 14(abstr.).

CRAIGIE, J.S. (1974). Storage Products. In: *Algal Physiology and Biochemistry* (Ed. by W.D.P. Stewart). pp. 206-235. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.

CZYGAN, F.-C. (1963). Untersuchungen über die Nitratreduktion der Grünalge *Ankistrodesmus braunii in vivo* und *in vitro*. *Planta.*, **60**, 225-242.

DAWSON, E.Y. (1944). A new parasitic red alga from southern California. *Bull. Torrey bot. Club.*, **71**, 655-657.

DAWSON, E.Y. (1966). *Marine Botany: An Introduction*. Holt, Rinehart and Winston, New York.

DIECKMANN, G.S. (1978). Aspects of growth and production of *Laminaria pallida* (Grev.) J. Ag. off the Cape Peninsula. MSc. thesis, University of Cape Town.

DIECKMANN, G.S. (1980). Aspects of the ecology of *Laminaria pallida* (Grev.) J. Ag. of the Cape Peninsula (South Africa). I. Seasonal growth. *Bot. mar.*, **23**, 579-585.

DISCHE, Z. (1947). A new specific colour reaction of hexuronic acids. *J. biol. Chem.*, **167**, 189-198.

DIXON, P.S. (1973). *Biology of the Rhodophyta*. Oliver and Boyd, Edinburgh.

DREW, E.A. (1969). Uptake and metabolism of exogenously supplied sugars by brown algae. *New Phytol.*, **68**, 35-43.

DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A. and SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Analyt. Chem.*, **28**, 350-356.

EMERSON, C.J., BUGGELN, R.G. and BAL, A.K. (1982). Translocation in *Saccorhiza dermatodea* (Laminariales, Phaeophyceae): anatomy and physiology. *Can. J. Bot.*, **60**, 2164-2184.

EPPLEY, R.W., COATSWORTH, J.L. and SOLORZANO, L. (1969). Studies on nitrate reductase in marine phytoplankton. *Limnol. Oceanogr.*, **14**, 194-205.

EPPLEY, R.W., PACKARD, T.T. and MACISAAC, J.J. (1970). Nitrate reductase in Peru current phytoplankton. *Mar. Biol.*, **6**, 195-199.

EVANS, L.V., CALLOW, J.A. and CALLOW, M.E. (1973). Structural and physiological studies on the parasitic red alga *Holmsella*. *New Phytol.*, **72**, 393-402.

EVANS, L.V., CALLOW, J.A. and CALLOW, M.E. (1978). Parasitic red algae: An appraisal. In: *Modern Approaches to the Taxonomy of Red and Brown Algae*. (Ed. by D.G.C. Irvine and J.H. Price). Systematics Association special volume no. 10, ch. 5, pp. 87-109. Academic Press, London, New York.

FAN, K.-C. (1961). Studies on *Hypneocolax*, with a discussion on the origin of parasitic red alga. *Nova Hedwigia*, **3**, 119-128.

FAN, K.-C. and PAPENFUSS, G.F. (1959). Red algal parasites occurring on members of the Gelidiales. *Madroño*, **15**, 33-38.

FAUST, H. (1967) Probenschemie ^{15}N-markierter Stickstoff-verbindungen im Mikro-bis Nanomolbereich für die emissions-spektrometrische Isotopenanalyse. *Isopenpraxis*, **3**, 100-103.

FELDMAN, J. and FELDMANN, G. (1958). Recherches sur quelques Floridées parasites. *Revue gén. Bot.*, **65**, 49-124.

FIELD, J.G., JARMAN, N.G., DIECKMANN, G.S., GRIFFITHS, C.L. VELIMIROV, B. and ZOUTENDYK, P. (1977). Sun, seaweeds, waves and lobsters. The dynamia of a west coast kelp bed. *S. Afr. J. Sci.*, **73**, 7-10.

FIELD, J.G., GRIFFITHS, C.L., LINLEY, E.A., CARTER, R.A. and ZOUTENDYK, P. (1980a). Upwelling in a nearshore marine ecosystem and its biological implications. *Estuar. coast. mar. Sci.*, **11**, 133-150.

FIELD, J.G., GRIFFITHS, C.L., GRIFFITHS, R.J., JARMAN, N.G., ZOUTENDYK, P., VELIMIROV, B. and BOWES, A. (1980b). Variation in structure and biomass of kelp communities along the south-western Cape coast. *Trans. R. Soc. S. Afr.*, **44**, 145-203.

FLOC'H, J.Y. and PENOT, M. (1978). Influence of cycloheximide on translocation of ^{32}P in *Laminaria digitata* (L.) Lamoroux. *Bot. mar.*, **21**, 5-11.

FRITSCH, F.E. (1945). *The Structure and Reproduction of the algae.* Vol. II. Cambridge University Press, Cambridge.

GAYLER, K.R. and MORGAN, W.R. (1976). An NADP-dependent glutamate dehydrogenase in chloroplasts from the marine green alga *Caulerpa simpliciuscula*. *Pl. Physiol.*, **58**, 283-287.

GOFF, L.J. (1976). The biology of *Harveyella mirabilis* (Cryptonemiales; Rhodophyceae). V. Host responses to parasite infection. *J. Phycol.*, **12**, 313-328.

GOFF, L.J. (1977). Developmental studies of the red algal adelphoparasite *Gardneriella tuberiflora* (Gigartinales). *J. Phycol.*, **13** (suppl.) 24(abstr.).

GOFF, L.J. and COLE, K. (1976). The biology of *Harveyella mirabilis* (Cryptonemiales, Rhodophyceae). III. Spore germination and the subsequent development within the host *Odonthalia floccosa* (Ceramiales, Rhodophyceae). *Can. J. Bot.*, **54**, 268-280.

GOVINDJEE and BRAUN, B.Z. (1974). Light absorption, emission and photosynthesis. In: *Algal Physiology and Biochemistry.* (Ed. by W.D.P. Stewart) pp. 346-390. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.

HARLIN, M.M. (1973a). "Obligate" algal epiphyte *Smithora naiadum* grows on a synthetic substrate. *J. Phycol.* **9**, 230-232.

HARLIN, M.M. (1973b). Transfer of products between epiphytic marine algae and host plants. *J. Phycol.*, **9**, 243-248.

HARLIN, M.M. and CRAIGIE, J.S. (1975). The distribution of photosynthate in *Ascophyllum nodosum* as it relates to epiphytic *Polysiphonia lanosum*. *J. Phycol.*, **11**, 109-113.

HARTMANN, T. and ESHRICH, W. (1969). Stofftransport in Rotalgen. *Planta*, **85**, 303-312.

HATCHER, B.G., CHAPMAN, A.R.O. and MANN, K.H. (1977). An annual carbon budget for the kelp *Laminaria longicruris*. *Mar. Biol.*, **44**, 85-96.

HATTORI, A. and MEYERS, J. (1966). Reduction of nitrate and nitrite by subcellular preparations of *Anabaena cylindrica*. I. Reduction of nitrite to ammonia. *Pl. Physiol.*, **41**, 1031-1036.

HATTORI, A. and MEYERS, J. (1967). Reduction of nitrate and nitrite by subcellular preparations of *Anabaena cylindrica*. II. Reduction of nitrate to nitrite. *Pl. Cell Physiol.*, **8**, 327-337.

HAUG, A., LARSEN, B. and SMIDSRØD, O. (1966). A study of the constitution of alginic acid by partial acid hydrolysis. *Acta chem. scand.*, **20**, 183-190.

HAUG, A., LARSEN, B. and SMIDSRØD, O. (1967a). Studies on the sequence of uronic acid residues in alginic acid. *Acts chem. scand.*, **21**, 691-704.

HAUG, A., MYKLESTAD, S., LARSEN, B. and SMIDSRØD, O. (1967b). Correlation between chemical structure and physical properties of alginates. *Acts chem. scand.*, **21**, 769-778.

HAXEN, P.G. and LEWIS, O.A.M. (1981). Nitrate assimilation in the marine kelp, *Macrocystis angustifolia* (Phaeophyceae). *Bot. mar.*, **14**, 631-635.

HAY, C.H., HODGSON, V., SCOTT, G. and MILLER, J. (1983). Colorimetric determination of the algin content of three South African kelp seaweeds (*Ecklonia maxima*, *Laminaria pallida* and *Macrocystis angustifolia*) *Trans. R. Soc. S. Afr.*, **45**, 73-89.

HELLEBUST, J.A. and HAUG, A. (1972). Photosynthesis, translocation and alginic acid synthesis in *Laminaria digitata* and *Laminaria hyperborea*. *Can. J. Bot.*, **50**, 169-176.

HEWITT, E.J. (1970). Physiological and biochemical factors controlling the assimilation of inorganic nitrogen supplies by plants. In: *Nitrogen Nutrition of the Plant*. (Ed. by E.A. Kirby) pp. 78-103. University of Leeds Press, Leeds.

HOLLIGAN, P.M. and DREW, E.A. (1971). Routine analysis by gas liquid chromatography of soluble carbohydrates in extracts of plant tissue. II. Quantitative analysis of standard carbohydrates and the separation and estimation of soluble sugars and polyols from a variety of plant tissues. *New Phytol.*, **70**, 271-297.

HOUGH, L., JONES, J.K.N. and WADEMAN, W.H. (1950). Quantitative analysis of mixtures of sugars by the method of partition chromatography. V. Improved methods of separation and detection of sugars and their methylated derivatives on the paper chromatograms. *J. chem. Soc. (Lond.)*, **7**, 1702-1706.

JOHNSTON, C.S., JONES, R.G. and HUNT, R.D. (1977). A seasonal carbon budget for a laminarian population in a Scottish sea loch. *Helgoländer wiss. Meeresunters.*, **30**, 527-545.

JENSEN, A., SUNDE, I. and HAUG, A. (1955). The Quantitative Determination of Alginic Acid. Rep. No. 12 Norwegian Inst. Seaweed Research, Trondheim.

KAISER, J.J. (1978). An investigation of the pathway of nitrogen incorporation into the leaf and root metabolism of *Helianthus annuus* L. MSc. thesis, University of Cape Town.

KAISER, J.J. and LEWIS, O.A.M. (1980). Nitrate-nitrogen assimilation in the leaves of *Helianthus annuus*. *New Phytol.*, **85**, 235-242.

KAMEN, M.D. (1957). *Isotopic Tracers in Biology*. Academic Press, New York.

KEDENBURG, C.P. (1971). A lithium buffer system for accelerated single-column amino acid analysis in physiological fluids. *Analyt. Biochem.*, **40**, 35-42.

KHAILOV, K.M. and BURLAKOVA, Z.P. (1969). Release of dissolved organic matter by marine seaweeds and distribution of their total organic production to inshore communities. *Limnol. Oceanog.*, **14**, 521-527.

KREMER, B.P. (1976a). Mannitol in the Rhodophyceae - A reappraisal. *Phytochem.*, **15**, 1135-1138.

KREMER, B.P. (1976b). ^{14}C-Assimilate pattern and kinetics of photosynthetic $^{14}CO_2$-assimilation of the marine alga *Bostrychia scorpioides*. *Planta*, **129**, 63-67.

KREMER, B.P. (1978a). Studies on $^{14}CO_2$-assimilation in marine Rhodophyceae. *Mar. Biol.*, **48**, 47-54.

KREMER, B.P. (1978b). Aspects of CO_2-fixation in some freshwater Rhodophyceae. *Phycologia*, **17**, 430-434.

KUGRENS, P. (1970). Comparative ultrastructure of several parasitic red alga. *J. Phycol.*, **6**, (suppl.) 9.

KUGRENS, P. and WEST, J.A. (1972a). Ultrastructure of spermatial development in the parasitic red alga *Levringiella gardeneri* and *Erythrocystis saccata*. *J. Phycol.*, **8**, 331-343.

KUGRENS, P. and WEST, J.A. (1972b). Ultrastructure of tetrasporogenesis in the parasitic red alga *Levringiella gardneri* (Setchell) Kylin. *J. Phycol.*, **8**, 370-383.

KUGRENS, P. and WEST, J.A. (1973). The ultrastructure of an alloparasitic red alga *Choreocolax polysiphoniae*. *Phycologia*, **12**, 175-186.

LEA, P.J. and MIFLIN, B.J. (1974). An alternative route for nitrogen assimilation in higher plants. *Nature (Lond.)*, **251**, 624-616.

LEA, P.J. and MIFLIN, B.J. (1979). Assimilation of ammonium nitrogen by chlorophyllous tissue. In: *Nitrogen Assimilation of Plants. Proc. 8th Long Ashton Symposium* (Ed. by E.J. Hewitt and C.V. Cutting) pp. 475-487. Academic Press, London and New York.

LEA, P.J. and NORRIS, R.D. (1976). The use of amino acid analogues in studies on plant metabolism. *Phytochem.*, **15**, 585-595.

LEWIS, D.H. (1974). Micro-organisms and plants:The evolution of parasitism and Mutalism. *Symposium of the Soc. Gen. Microbiol.*, XXIV. 367-392. Evolution in the Microbiol. World.

LEWIS, D.H. and HARLEY, J.S. (1965). Carbohydrate physiology of mycorrhiza roots of beech. I. Identity of endogenous sugars and utilization of exogenous sugars. *New Phytol.*, **64**, 225-237.

LEWIS, D.H. and SMITH, D.C. (1967a). Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. *New Phytol.*, **66**, 143-184.

LEWIS, D.H. and SMITH, D.C. (1967b). Sugar alcohols (polyols) in fungi and green plants. II. Methods of detection and quantitative estimation in plant extracts. *New Phytol.*, **66**, 185-204.

LEWIS, D.H., CHEN, C., WOODS, G. and CULPIN, L.A. (1972). A modified p-anisidine technique for the detection and permanent recording of free and combined fructose on paper chromatograms. *J. Chromat.*, **74**, 369-372.

LEWIS, O.A.M. and PROBYN, T.A. (1978). ^{15}N-Incorporation and glutamine synthetase inhibition studies of nitrogen assimilation in leaves of the nitrophile *Datura stramonium* L. *New Phytol.*, **81**, 519-526.

LINLEY, E.A.S., NEWELL, R.C. and BOSMA, S.A. (1981). Heterotrophic utilization of mucilage released during fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). I. Development of microbiol communities associated with the degradation of kelp mucilage. *Mar. Ecol. Prog. Ser.*, **4**, 31-41.

LINSKENS, H.F. (1963). Beitrag zur Frage der Beziehung zwischen Epiphyt und Basiphyt bei marinen Algen. *Pubbl. Staz. zool. Napoli.*, **33**, 274-293.

LOBBAN, C.S. (1974). A simple rapid method of solubilizing algal tissue for scintillation counting. *Limnol. Oceanogr.*, **19**, 356-359.

LOBBAN, C.S. (1978). Translocation of ^{14}C in *Macrocystis integrifolia* (Phaeophyceae) *J. Phycol.*, **14**, 178-182.

LOSADA, M., RAMIREZ, J.M., PANEQUE, A. and DEL CAMPO, F.F. (1965). Light and dark reduction of nitrate in a reconstituted chloroplast system. *Biochem. biophys. Acta.*, **109**, 86-96.

LOSADA, M., PANEQUE, A., APARICHIO, P.J., VEGA, J.M., CÁRDENAS, J. and HERRERA, J. (1970). Inactivation and repression by ammonium of the nitrate reducing system in *Chlorella*. *Biochem. biophys. Res. Commun.*, **38**, 1009-1015.

LUCAS, M.I., NEWELL, R.C. and VELIMIROV, B. (1981). Heterotrophic utilization of mucilage released during fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). II. Differential utilization of dissolved organic components from kelp mucilage. *Mar. Ecol. Prog. Ser.*, **4**, 43-55.

LÜNING, K., SCHMITZ, K. and WILLENBRINK, J. (1972). Translocation of ^{14}C-labelled assimilates of two *Laminaria* species. *Proc. Int. Seaweed Symp.*, **7**, 420-425.

LÜNING, K., SCHMITZ, K. and WILLENBRINK, J. (1973). CO_2 fixation and translocation in benthic marine algae. III. Rates and ecological significance of translocation in *Laminaria hyperborea* and *L. saccharina*. *Mar. Biol.*, **23**, 275-281.

MACKIE, W. and PRESTON, R.D. (1974). Cell wall and intercellular region polysaccharides. In: *Algal Physiology and Biochemistry*. (Ed. by W.D.P. Stewart) pp. 40-85. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.

MAJAK, W., CRAIGIE, J.S. and McLACHLAN, J. (1966). Photosynthesis in algae. I. Accumulation products in the Rhodophyceae. *Can. J. Bot.*, **44**, 541-549.

MANN, K.H., JARMAN, N.G. and DIECKMANN, G.S. (1979). Development of a method for measuring the productivity of *E. maxima* (Osbeck) Papenf. *Trans. R. Soc. S. Afr.*, **44**, 27-42.

MARTIN, M.T. and POCOCK, M.A. (1953). South African parasitic Florideae and their hosts. 2. Some South African parasitic Florideae. *J. Linn. Soc. (Bot.)*, **55**, 48-64.

MARTINEZ NADAL, N.G., RODRIGUEZ, L.V. and CASILAS, C. (1963). Low molecular weight carbohydrates in *Sargassum natans* from Puerto Rico. *J. Pharm. Sci.*, **52**, 498.

* MATEUS, H., REGENSTEIN, J.M. and BAKER, R.C. (1977). Studies to improve the extraction of mannitol from *Macrocystis pyrifera*, a marine brown algae. *Econ. Bot.*, **31**, 21-27.

MIFLIN, B.J. and LEA, P.J. (1976). The pathway of nitrogen assimilation in plants. *Phytochem.*, **15**, 873-885.

MIFLIN, B.J. and LEA, P.J. (1977). Amino acid metabolism. *A. Rev. Pl. Physiol.*, **28**, 299-329.

MOEBUS, K. and JOHNSON, K.M. (1974). Exudation of dissolved organic compounds by marine algae. *Mar. Biol.*, **26**, 117-125.

MOEBUS, K., JOHNSON, K.M. and SIEBURTH, J.McN. (1974). Rehydration of dessicated intertidal brown algae:release of dissolved organic carbon and water uptake. *Mar. Biol.*, **26**, 127-134.

MORRIS, I. (1974). Nitrogen assimilation and protein synthesis. In: *Algal Physiology and Biochemistry*. (Ed. by W.D.P. Stewart). pp. 583-609. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.

MUNDA, I. (1967). Der Einfluss der Salinität auf die chemische Zusammensetzung, das Wachstum und die Fruktifidation einiger Fucaceen. *Nova Hedwigia*, **13**, 471-508.

* NEWELL, R.C., LUCAS, M.I., VELIMIROV, B. and SEIDERER, L.J. (1980). Quantitative significance of dissolved organic losses following fragmentation of kelp (*Ecklonia maxima* and *Laminaria pallida*). *Mar. Ecol. Prog. Ser.*, **2**, 45-59.

NEWELL, R.C., FIELD, J.G. and GRIFFITHS, C.L. (1982). Energy balance and the significance of micro-organisms in a kelp bed community. *Mar. Ecol. Prog. Ser.*, **8**, 103-113.

NICHOLSON, N.L. and BRIGGS, W.R. (1972). Translocation of photosynthate in the brown alga *Nereocystis*. *Am. J. Bot.*, **59**, 97-106.

NONOMURA, A.M. (1979). Development of *Janczewskia morimotoi* (Ceramiales) on its host *Laurencia nipponica* (Ceramiales, Rhodophyceae). *J. Phycol.*, **15**, 154-162.

OLIVER, F.W. (1887). On the obliteration of the sieve tube in the Laminarieae. *Ann. Bot.*, **1**, 95-117.

OSAJIMA, Y. and YAMAFUJI, K. (1964). Reduction of nitrate to ammonia by enzymes isolated from green algae. *Enzymologia*, **27**, 129-140.

PARKER, B.C. (1963). Translocation in the giant kelp *Macrocystis*. *Science, N.Y.*, **140**, 891-892.

PARKER, B.C. (1965). Translocation in the giant kelp *Macrocystis*. I. Rates, direction, quantity of ^{14}C-labelled products and fluorescein. *J. Phycol.*, **1**, 41-46.

PARKER, B.C. (1966). Translocation in *Macrocystis*. III. Composition of sieve tube exudate and identification of the major ^{14}C-labelled products. *J. Phycol.*, **2**, 38-41.

PARKER, B.C. (1971). Studies on translocation in *Macrocystis*. *Nova Hedwigia*, **32**, 191-195.

PARKER, B.C. and HUBER, J. (1965). Translocation in *Macrocystis*. II. Fine structure of the sieve tubes. *J. Phycol.*, **1**, 172-179.

PARKER, J. and PHILPOTT, D.E. (1961). The ultrastructure of sieve plates of *Macrocystis pyrifera*. *Bull. Torrey bot. Club*, **88**, 85-90.

PERCIVAL, E. (1968). Marine algal carbohydrates. *Oceanog. Mar. Biol. Ann. Rev.*, **6**, 137-161.

PERCIVAL, E. (1978). Do the polysaccharides of brown and red seaweeds ignore taxonomy? In: *Modern Approaches to the Taxonomy of Red and Brown Algae*. (Ed. by D.G.C. Irvine and J.H. Price) Systematics Association special volume no. 10, ch. 3, pp. 47-62. Academic Press, London, New York.

PERCIVAL, E. and McDOWELL, R.H. (1967). *Chemistry and Enzymology of Marine Algal Polysaccharides*. Academic Press, London and New York.

POMPOWSKI, T. and TROKOWICZ, D. (1966). Determination of mannitol in the Baltic *Fucus* (changes in the mannitol content depending on the season). *Przem. Spozyw.*, **20**, 491-492.

POWELL, J.H. and MEEUSE, B.J.D. (1964). Laminaria in some Phaeophyta of the Pacific coast. *Econ. Bot.*, **18**, 164-166.

PROBYN, T.A. (1978). The pathway of nitrogen assimilation in *Datura stramonium* L. MSc. thesis, University of Cape Town.

PROBYN, T.A. and LEWIS, O.A.M. (1979). The route of nitrate-nitrogen assimilation in the root of *Datura stramonium* L. *J. exp. Bot.*, **30**, 299-305.

RAO, M.U. (1969). Seasonal variations in growth, alginic acid and mannitol contents of *Saragassum wightii* and *Turbinaria conoides*. *Proc. Int. Seaweed Symp.*, **6**, 579-584.

RAWLENCE, D.J. (1972). An ultrastructural study of the relationship between rhizoids of *P. lanosa* (L.) Tandy (Rhodophyceae) and tissue of *Ascophyllum nodosum* (L.) Le Jolis (Phaeophyceae). *Phycologia*, **11**, 279-290.

RAWLENCE, D.J. and TAYLOR, A.R.A. (1970). The rhizoids of *Polysiphonia lanosa*. *Can. J. Bot.*, **48**, 607-611.

REES. D.A., (1969). Structure, conformation and mechanism in the formation of polysaccharide gels and networks. *Advance Carbohyd. Res.*, **32**, 195-201.

REYES-VASQUEZ, G. (1970). Studies on the diatom flora living on *Thalassia testudinium* Konig in Biscayne Bay, Florida. *Bull. Mar. Sci.*, **20**, 105-134.

RIGG, G.B. (1925). Some physiology of the sieve tubes of *Nereocystis*. *Publ. Puget Sound Biol. Stat.*, **3**, 311-329.

SARGENT, M.C. and LANTRIP, L.W. (1952). Photosynthesis, growth and translocation in a giant kelp. *Am. J. Bot.*, **39**, 99-107.

SCHAFFER, J., BAKER, J.E. and THOMPSON, J.F. (1961). A *Chlorella* mutant lacking nitrate reductase. *Am. J. Bot.*, **48**, 896-899.

SCHMITZ, K. and LOBBAN, C.S. (1976). A survey of translocation in Laminariales (Phaeophyceae). *Mar. Biol.*, **36**, 207-216.

SCHMITZ, K. and SRIVASTAVA, L.M. (1974). Fine structure of sieve tubes in *Laminaria groenlandica* Rosenv. *Cytobiol.*, **10**, 66-87.

SCHMITZ, K. and SRIVASTAVA, L.M. (1975). On the fine structure of sieve tubes and the physiology of assimilate transport in *Alaria marginata* Postels and Ruprecht. *Can. J. Bot.*, **53**, 861-876.

SCHMITZ, K. and SRIVASTAVA, L.M. (1976). The fine structure of sieve elements in *Nereocystis luetkeana*. *Am. J. Bot.*, **63**, 679-693.

SCHMITZ, K. and SRIVASTAVA, L.M. (1979a). Long distance transport in *Macrocystis integrifolia*. I. Translocation of ^{14}C-labelled assimilates. *Pl. Physiol.*, **63**, 995-1002.

SCHMITZ, K. and SRIVASTAVA, L.M. (1979b). Long distance transport in *Macrocystis integrifolia*. II. Tracer experiments with ^{14}C and ^{32}P. *Pl. Physiol.*, **63**, 1003-1009.

SCHMITZ, K., LÜNING, K. and WILLENBRINK, J. (1972). CO_2-Fixierung und Stofftransport in benthischen marinen Algen. II. Zum Ferntransport ^{14}C-markierter Assimilate bei *Laminaria hyperborea* und *Laminaria saccharine*. *Z. PflPhysiol.*, **67**, 418-429.

SCHWEIGER, R.G. (1967). Low molecular weight compounds in *Macrocystis pyrifera* a marine alga. *Archs. Biochem. Biophys.*, **118**, 383-387.

SETCHELL, W.A. (1918). Parasitism among the red algae. *Proc. Am. phil. Soc.*, **57**, 155-172.

SIDEMAN, E.J. and SCHEIRER, D.C. (1977). Some fine structural observations on developing and mature sieve elements in the brown alga *Laminaria saccharina*. *Am. J. Bot.*, **64**, 649-657.

SIEBURTH, J. McN. (1969). Studies on the algal substances in the sea. 3. The production of extracellular organic matter by littoral marine alga. *J. exp. mar. Biol. Ecol.*, **3**, 290-309.

* SIMONS, R.H. (1976). Seaweeds of Southern Africa: Guide lines for their study and identification. *Fish. Bull. S. Afr.*, **7**, 1-113.

SIMS, A.P. and FOLKES, B.F. (1964). A kinetic study of the assimilation of ^{15}N-ammonia and the synthesis of amino acids in an experimentally growing culture of *Candida utilis*. *Proc. R. Soc. B.*, **159**, 479-502.

SOMOGYI, M. (1945). Determination of blood sugars. *J. biol. Chem.*, **160**, 69-73.

SOUTH, R.G. (1974). *Herpodiscus* gen. nov. and *Herpodiscus durvilleae* (Lindauer) comb. nov., a parasite of *Durvillaea antartica* (Chamisso) Hariot endemic to New Zealand. *J. R. Soc. N.Z.*, **4**, 455-461.

STEINBIß, H-H. and SCHMITZ, K. (1973). CO_2-Fixierung und Stofftransport in benthischen marinen Algen. V. Zur autoradiographischen Lokalisation der Assimilattransport in Thallus von *Laminaria hyperborea*. *Planta*, **112**, 253-263.

STEPHEN, A.M. (1980). Plant carbohydrates. In: Encyclopaedia of Plant Physiology New Series, Vol. 8, *Secondary Plant Products* (Ed. by E.A. Bell and B.V. Charlwood) pp. 555-584. Springer-Verlag, Berlin.

STEPHENSON, R.A., BROWN, R.H. and ASHLEY, D.A. (1976). Translocation of ^{14}C-labelled assimilate and photosynthesis in C_3 and C_4 species. *Crop. Sci.*, **16**, 285-288.

STUART, V., NEWELL, R.C. and LUCAS, M.L. (1981). Heterotrophic utilization of particulate matter from the kelp *Laminaria pallida*. *Mar. Ecol. Prog. Ser.*, **4**, 337-348.

STURCH, H.H. (1926). *Choreocolax polysiphoniae* Reinsch. *Ann. Bot.*, **40**, 585-605.

SWEELEY, C.C., BENTLEY, R., MAKITA, M. and WELLS, W.W. (1963). Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J. Am. Chem. Soc.*, **85**, 2497-2507.

SYKES, M.G. (1908). Anatomy and histology of *Macrocystis pyrifera* and *Laminaria saccharina*. *Ann. Bot.*, **22**, 291-325.

SYRETT, P.J. (1962). Nitrogen assimilation. In: *Physiology and Biochemistry of Algae*. (Ed. by W.D.P. Stewart). pp. 171-188. Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne.

TATE, S.S. and MEISTER, A. (1973). Glutamine synthetases of mammalian liver and brain. In: *The Enzymes of Glutamine Metabolism*. (Ed. by S. Prusiner and E.R. Stadtman) pp. 77-127. Academic Press, New York.

TREVELYAN, W.E., PROCTOR, D.P. and HARRIS, J.S. (1950). Detection of sugars on paper chromatograms. *Nature (Lond.)* **166**, 444-445

TURNER, C.H.C. and EVANS, L.V. (1977). Physiological studies on the relationship between *Ascophyllum nodosum* and *Polysiphonia lanosa*. *New Phytol.*, **79**, 363-376.

TURNER, C.H.C. and EVANS, L.V. (1978). Translocation of photoassimilated ^{14}C in the red alga *Polysiphonia lanosa*. *Br. Phycol. J.*, **13**, 51-55.

VEGA, J.M., HERRERA, J., APARICHIO, P.J., PANEQUE, A. and LOSADA, M. (1971). Role of molybdenum in nitrate reduction by *Chlorella*. *Pl. Physiol.*, **48**, 294-229.

VELIMIROV, B., FIELD, J.G., GRIFFITHS, C.L. and ZOUTENDYK, P. (1977). The ecology of kelp bed communities in the Benguela upwelling system. Analysis of biomass and spatial distribution. *Helgoländer wiss. Meeresunters.*, **30**, 495-518.

VON HOLT, M.M., LIGTHELM, S.P. and NUNN, J.R. (1955). South African seaweeds:Seasonal variations in the chemical composition of some Phaeophyceae. *J. Sci. Food. Agric.*, **6**, 193-197.

WENT, J.L. and TAMMES, P.M.L. (1973). Organelles in the medulla exudate of *Laminaria*. *Acta. bot. neerl.*, **22**, 730-731.

YAMAGUCHI, T., IKAWA, T. and NISIZAWA, K. (1966). Incorporation of radioactive carbon from $H^{14}CO_3^-$ into sugar constituents by a brown alga, *Eisena bicyclis*, during photosynthesis and its fate in the dark. *Pl. Cell Physiol.*, **7**, 217-229.

YAMAGUCHI, T. IKAWA, T. and NISIZAWA, K. (1969). Pathway of mannitol formation during photosynthesis in brown algae. *Pl. Cell Physiol., Tokyo*, **10**, 425-440.

ZIEGLER, H. (1963). Untersuchungen über die Feinstrucktur des Phloems. II. Mitteilung:Die Siebplatten bei der Braunalge *Macrocystis pyrifera* (L.). *Protoplasma*, **57**, 779-786.

ZIEGLER, H. and RUCK, I. (1967). Untersuchungen über die Feinstrucktur des Phloems. III. Mitteilung:Die "Trompetezellen" von *Laminaria* Arter. *Planta*, **73**, 62-73.

ZUMFT, W.G., PANEQUE, A., APARICHIO, P.J. and LOSADA, M. (1969). Mechanism of nitrate reduction in *Chlorella*. *Biochem. biophys. Res. Commun.*, **36**, 980-986.

APPENDIX TABLE 1

Laminaria pallida: The distribution of ^{14}C-labelled assimilates along the lamina after one hour photosynthesis in ^{14}C-labelled seawater. Each value is a mean of four replicates from each of six plants ± SEM. Results are expressed as ^{14}C-activity in DPM x $10^3 mm^{-2}$

Distance from the area of the blade exposed to ^{14}C (mm)	^{14}C-activity DPM x $10^3 mm^{-2}$ ± SEM
10	12,2 ± 0,7
20	10,7 ± 0,4
40	9,4 ± 0,4
60	6,8 ± 0,4
80	3,7 ± 0,9
100	1,7 ± 0,8
120	1,2 ± 0,5
140	0,3 ± 0,2
160	0,2 ± 0,2

APPENDIX TABLE 2

Ecklonia maxima: The distribution of ^{14}C-labelled assimilates along the lamina after one hour photosynthesis in ^{14}C-labelled seawater. Each value is a mean of four replicates from each of six plants ± SEM. Results are expressed as ^{14}C-activity in DPM x 10^{3} mm^{-2} ± SEM.

Distance from the area of the blade exposed to ^{14}C (mm)	^{14}C-activity DPM x 10^{3} mm^{-2} ± SEM
50	14,9 ± 0,7
100	14,3 ± 1,0
150	14,2 ± 0,4
200	14,6 ± 0,7
220	14,8 ± 0,7
240	11,8 ± 2,2
260	12,0 ± 2,3
280	9,5 ± 3,1
300	2,2 ± 1,8
320	0,3 ± 0,2
340	0,2 ± 0,2
360	0,2 ± 0,2

APPENDIX TABLE 3

Seasonal variation of mannitol in the frond, meristematic region and stipe of *L. pallida*. Each value is a mean of sixteen replicates ± SEM.

Time of Sampling		Concentration of mannitol mg g^{-1} dry mass ± SEM		
		frond	meristematic region	stipe
July	1978	63,5 ± 1,56	31,2 ± 1,24	43,1 ± 1,24
August	1978	71,0 ± 1,36	33,2 ± 1,24	46,0 ± 1,28
September	1978	66,1 ± 1,46	30,6 ± 1,16	46,0 ± 1,12
October	1978	59,9 ± 1,28	31,1 ± 1,24	50,6 ± 1,12
November	1978	49,5 ± 1,22	32,2 ± 1,14	48,9 ± 1,20
December	1978	47,3 ± 1,24	32,2 ± 1,20	42,8 ± 1,12
January	1979	42,7 ± 1,36	33,8 ± 1,30	49,1 ± 1,40
February	1979	39,1 ± 1,20	31,9 ± 1,26	49,4 ± 1,32
March	1979	43,3 ± 1,30	33,0 ± 1,36	49,4 ± 1,20
April	1979	37,8 ± 1,24	31,4 ± 1,32	38,2 ± 1,28
May	1979	46,7 ± 1,22	35,4 ± 1,20	44,8 ± 1,26
June	1979	64,4 ± 1,36	35,4 ± 1,24	49,7 ± 1,14
July	1979	78,6 ± 1,64	32,0 ± 1,28	39,9 ± 1,92
August	1979	75,3 ± 1,50	33,0 ± 1,20	48,0 ± 1,24
September	1979	68,1 ± 1,22	32,1 ± 1,12	46,4 ± 1,44
October	1979	55,3 ± 1,30	31,4 ± 1,18	45,5 ± 1,26
November	1979	53,4 ± 1,30	32,5 ± 1,20	47,2 ± 1,26
December	1979	44,8 ± 1,20	30,7 ± 1,24	46,5 ± 1,16

APPENDIX TABLE 4

Seasonal variation of mannitol in the frond, meristematic region and stipe of *E. maxima*. Each value is a mean of sixteen replicates ± SEM.

Time of Sampling		Concentration of mannitol mg g^{-1} dry mass ± SEM		
		frond	meristematic region	stipe
July	1978	94,3 ± 1,56	37,5 ± 1,22	62,4 ± 1,32
August	1978	98,9 ± 1,12	37,3 ± 1,28	63,2 ± 1,14
September	1978	94,4 ± 1,24	39,6 ± 1,30	63,3 ± 1,26
October	1978	81,5 ± 1,14	38,3 ± 1,34	63,5 ± 1,20
November	1978	79,9 ± 1,22	34,1 ± 1,46	63,4 ± 1,32
December	1978	64,7 ± 1,18	37,3 ± 1,14	63,6 ± 1,20
January	1979	72,3 ± 1,30	40,4 ± 1,22	63,4 ± 1,26
February	1979	64,0 ± 1,28	39,8 ± 1,24	63,1 ± 1,19
March	1979	63,9 ± 1,18	39,1 ± 1,26	63,3 ± 1,26
April	1979	63,9 ± 1,36	39,0 ± 1,18	66,2 ± 1,26
May	1979	63,8 ± 1,16	39,0 ± 1,28	68,1 ± 1,26
June	1979	63,5 ± 1,30	39,3 ± 1,24	74.9 ± 1,26
July	1979	94,7 ± 1,30	37,6 ± 1,36	59,6 ± 1,28
August	1979	101,1 ± 1,47	37,9 ± 1,20	63,0 ± 1,36
September	1979	94,7 ± 1,16	38,9 ± 1,20	61,3 ± 1,44
October	1979	86,8 ± 1,20	36,3 ± 1,32	62,9 ± 1,24
November	1979	85,1 ± 1,26	39,4 ± 1,24	59,2 ± 1,34
December	1979	69,2 ± 1,52	38,9 ± 1,32	61,0 ± 1,26

APPENDIX TABLE 5

Seasonal variation of laminaran in the frond, meristematic region and stipe of *L. pallida*. Each value is a mean of sixteen replicates ± SEM.

Time of Sampling		Concentration of laminaran mg g^{-1} dry mass ± SEM		
		frond	meristematic region	stipe
July	1978	18,6 ± 1,52	9,1 ± 0,62	5,2 ± 0,22
August	1978	11,5 ± 1,00	11,9 ± 0,26	4,9 ± 0,14
September	1978	16,8 ± 1,06	11,4 ± 0,42	4,5 ± 0,16
October	1978	6,0 ± 1,54	10,9 ± 0,42	3,2 ± 0,22
November	1978	10,5 ± 1,34	9,7 ± 0,46	4,2 ± 0,34
December	1978	12,1 ± 1,46	9,5 ± 0,64	3,2 ± 0,16
January	1979	10,4 ± 1,24	8,4 ± 0,56	3,2 ± 0,20
February	1979	15,0 ± 1,22	11,3 ± 0,60	2,8 ± 0,12
March	1979	11,2 ± 1,36	9,9 ± 0,28	3,9 ± 0,20
April	1979	19,7 ± 1,46	8,4 ± 0,50	2,8 ± 0,10
May	1979	23,9 ± 1,44	8,5 ± 0,32	3,4 ± 0,18
June	1979	24,3 ± 1,48	8,0 ± 0,32	3,8 ± 0,24
July	1979	19,8 ± 1,34	10,0 ± 0,20	4,5 ± 0,10
August	1979	13,2 ± 1,38	8,3 ± 0,26	3,1 ± 0,28
September	1979	20,3 ± 1,54	7,9 ± 0,22	3,8 ± 0,32
October	1979	7,3 ± 1,44	8,6 ± 0,18	4,0 ± 0,30
November	1979	9,3 ± 1,32	9,5 ± 0,16	3,6 ± 0,24
December	1979	6,3 ± 1,36	9,9 ± 0,44	4,0 ± 0,30

APPENDIX TABLE 6

Seasonal variation of laminaran in the frond, meristematic region and stipe of *E. maxima*. Each value is a mean of sixteen replicates ± SEM.

Time of Sampling		Concentration of laminaran mg g^{-1} dry mass ± SEM		
		frond	meristematic region	stipe
July	1978	76,9 ± 1,26	13,5 ± 0,20	6,3 ± 0,30
August	1978	39,7 ± 1,04	13,5 ± 0,62	6,9 ± 0,16
September	1978	56,7 ± 1,64	13,2 ± 0,26	6,1 ± 0,22
October	1978	26,4 ± 1,60	12,6 ± 0,32	5,9 ± 0,14
November	1978	19,5 ± 1,64	12,1 ± 0,44	5,4 ± 0,08
December	1978	20,6 ± 1,74	12,3 ± 0,25	5,1 ± 0,12
January	1979	17,0 ± 1,46	11,4 ± 0,68	4,7 ± 0,16
February	1979	25,2 ± 1,30	12,3 ± 0,72	4,8 ± 0,14
March	1979	19,0 ± 1,26	12,2 ± 1,46	3,6 ± 0,14
April	1979	61,4 ± 1,42	12,3 ± 0,56	4,5 ± 0,10
May	1979	85,4 ± 1,66	11,5 ± 0,20	5,5 ± 0,20
June	1979	89,9 ± 1,52	15,7 ± 0,66	4,4 ± 0,20
July	1979	69,3 ± 1,50	14,5 ± 0,56	4,9 ± 0,26
August	1979	49,9 ± 1,72	12,5 ± 0,52	4,9 ± 0,12
September	1979	29,0 ± 1,42	14,0 ± 0,36	5,6 ± 0,21
October	1979	20,0 ± 1,46	15,2 ± 0,54	5,0 ± 0,40
November	1979	20,4 ± 1,56	13,9 ± 0,46	5,3 ± 0,50
December	1979	15,5 ± 1,52	14,0 ± 0,58	5,8 ± 0,14

APPENDIX TABLE 7

Seasonal variation of alginic acid in the frond, meristematic region and stipe of *L. pallida*. Each value is a mean of sixteen replicates ± SEM.

Time of Sampling		Concentration of alginic acid mg g^{-1} dry mass ± SEM		
		frond	meristematic region	stipe
July	1978	195,7 ± 1,36	187,0 ± 1,64	210,7 ± 2,28
August	1978	240,0 ± 1,32	194,4 ± 1,58	202,4 ± 1,40
September	1978	240,9 ± 1,18	212,4 ± 1,04	220,6 ± 0,26
October	1978	230,3 ± 1,54	198,4 ± 2,02	204,6 ± 1,76
November	1978	221,2 ± 2,38	213,3 ± 1,62	217,1 ± 1,56
December	1978	229,7 ± 0,58	222,5 ± 0,68	214,7 ± 1,26
January	1979	270,0 ± 0,56	205,2 ± 1,24	201,3 ± 0,46
February	1979	223,2 ± 0,94	191,2 ± 1,26	204,3 ± 0,96
March	1979	225,0 ± 1,84	214,2 ± 2,06	213,1 ± 0,68
April	1979	235,1 ± 2,00	208,8 ± 1,96	207,4 ± 0,88
May	1979	223,7 ± 1,72	209,0 ± 0,96	212,6 ± 1,66
June	1979	224,6 ± 1,36	196,9 ± 1,26	211,6 ± 1,60
July	1979	224,4 ± 1,64	211,3 ± 1,92	208,8 ± 1,84
August	1979	206,9 ± 1,78	191,1 ± 1,86	208,6 ± 1,64
September	1979	215,4 ± 1,52	221,7 ± 2,30	207,8 ± 1,06
October	1979	211,9 ± 2,12	214,0 ± 2,02	214,2 ± 1,60
November	1979	241,7 ± 2,80	227,3 ± 1,10	224,0 ± 1,70
December	1979	263,8 ± 1,29	225,4 ± 1,86	237,4 ± 1,62

APPENDIX TABLE 8

Seasonal variation of alginic acid in the frond, meristematic region and stipe of *E. maxima*. Each value is a mean of sixteen replicates ± SEM.

Time of Sampling		Concentration of alginic acid mg g^{-1} dry mass ± SEM		
		frond	meristematic region	stipe
July	1978	252,6 ± 1,36	213,1 ± 2,40	216,1 ± 1,56
August	1978	237,4 ± 4,08	224,4 ± 0,86	228,9 ± 3,08
September	1978	271,0 ± 1,60	205,5 ± 1,42	220,5 ± 0,20
October	1978	233,4 ± 1,24	223,0 ± 1,08	255,5 ± 2,26
November	1978	287,3 ± 1,52	227,6 ± 1,50	252,5 ± 2,54
December	1978	347,6 ± 0,62	235,6 ± 1,32	248,2 ± 3,08
January	1979	223,7 ± 1,90	233,3 ± 1,08	236,3 ± 1,78
February	1979	299,4 ± 1,34	215,5 ± 1,54	256,5 ± 1,16
March	1979	288,9 ± 1,08	227,7 ± 0,96	216,8 ± 1,84
April	1979	247,1 ± 0,96	208,6 ± 1,76	244,5 ± 2,14
May	1979	235,3 ± 1,64	204,3 ± 1,80	232,5 ± 1,50
June	1979	215,8 ± 1,88	208,9 ± 1,22	204,2 ± 1,04
July	1979	223,4 ± 1,68	186,7 ± 1,88	219,3 ± 2,54
August	1979	251,6 ± 2,02	209,9 ± 2,76	219,8 ± 2,48
September	1979	234,0 ± 1,76	207,7 ± 1,64	222,2 ± 1,86
October	1979	262,1 ± 1,54	217,3 ± 2,46	236,3 ± 1,54
November	1979	283,7 ± 1,88	232,6 ± 1,62	233,0 ± 2,08
December	1979	302,4 ± 1,16	224,2 ± 1,62	254,4 ± 1,70

APPENDIX TABLE 9

The abbreviations used in Tables 5, 6, 7 and 8 are given in full.

Abbreviations	Amino Compound
Ala	Alanine
Asg	Asparagine
Asp	Aspartic acid
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
Ser	Serine
Thr	Threonine
NH_3	Ammonium

APPENDIX TABLE 10

A time course of the ^{14}C-labelled assimilates, after incubation in $NaH^{14}CO_3$, in *C. minima*. A = *C. minima*. B = combined samples of whole plants of *C. minima* and the portions of stipe of *L. pallida* to which they were attached. C = stipe of *L. pallida* with no attached red algae. Each value is a mean of six replicates ± SEM.

Time in hours	^{14}C-Activity DPM x 10^3 g^{-1} dry mass ± SEM		
	A	B	C
2	9,5 ± 0,3	1,8 ± 0,4	0,3 ± 0,2
4	10,9 ± 0,4	2,8 ± 0,2	0,9 ± 0,2
6	11,9 ± 0,3	2,6 ± 0,2	1,3 ± 0,2
8	11,4 ± 0,4	3,1 ± 0,3	0,8 ± 0,3
10	11,2 ± 0,3	3,6 ± 0,2	1,4 ± 0,3
12	11,6 ± 0,5	3,2 ± 0,3	1,7 ± 0,2
14	10,9 ± 0,2	3,2 ± 0,2	1,4 ± 0,2
16	10,3 ± 0,4	2,9 ± 0,2	1,9 ± 0,2

APPENDIX TABLE 11

A time course of the ^{14}C-labelled assimilates, after incubation in $NaH^{14}CO_3$ in seawater, in *S. vittata*. A = *S. vittata*. B = combined samples of whole plants of *S. vittata* and the portions of the stipe of *E. maxima* to which they were attached. C = stipe of *E. maxima* with no attached red algae. Each value is a mean of six replicates ± SEM.

Time in hours	^{14}C-Activity DPM x 10^3 g^{-1} dry mass ± SEM		
	A	B	C
2	16,1 ± 0,5	2,1 ± 0,7	0,4 ± 0,3
4	17,8 ± 0,6	3,5 ± 0,4	0,9 ± 0,5
6	18,8 ± 0,5	3,2 ± 0,3	0,8 ± 0,2
8	17,3 ± 1,3	3,6 ± 0,2	0,7 ± 0,2
10	18,4 ± 0,7	3,9 ± 0,2	0,6 ± 0,2
12	19,0 ± 0,4	3,6 ± 0,2	0,8 ± 0,2
14	19,8 ± 1,4	4,2 ± 0,4	1,1 ± 0,3
16	19,1 ± 0,5	3,5 ± 0,5	0,9 ± 0,2

APPENDIX TABLE 12

The number of *C. minima* plants growing attached to $50cm^2$ of *L. pallida* stipe. Each figure is a mean of four $50cm^2$ regions chosen at random on the stipe of twenty individual plants with a total length of ± 1m.

Time of Year	Mean number of *C. minima* plants attached to a $50cm^2$ portion of *L. pallida* stipe ± SEM
August 1979	0,12 ± 0,27
September 1979	0,53 ± 0,21
October 1979	0,92 ± 0,38
November 1979	1,57 ± 0,26
December 1979	2,10 ± 0,42
January 1980	3,04 ± 0,66
February 1980	2,36 ± 0,84
March 1980	3,00 ± 0,53
April 1980	3,90 ± 0,42
May 1980	3,71 ± 0,37
June 1980	2,12 ± 0,25
July 1980	0,93 ± 0,14
August 1980	0,15 ± 0,20